RIJKSUNIVERSITEIT GRONINGEN

MANAGEMENT OF PEMPHIGUS

PROEFSCHRIFT

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Chapter I

General introduction

Pemphigus, derived from the term "pemphigoides pyertoi" used by Hippocrates (460-370 BC) to describe fever associated with blisters, refers to a group of autoimmune intraepidermal bullous diseases of the skin and mucous membranes. In Pemphigus vulgaris, the most common subtype, cutaneous lesions can be localized or generalized and usually present primarily as flaccid vesicles or bullae varying in size from less than 1 cm to several cm. The scalp, presternal, axillae and groin are predilection sites of involvement. The blisters may develop on normal skin and on erythematous macules or plaques and the fluid content is initially clear (serous). The blisters rupture easily and produce painful raw erosions. Pain of the blisters and erosions and the psychological loathing of a damaged skin, are important for patients suffering from pemphigus. Nikolsky's sign is a typical clinical phenomenon in pemphigus: sheetlike removal of epidermis by pushing with a finger. The clinical diagnosis is funded by laboratory investigations. Histopathology is used to determine acantholysis in the epidermis in a skin biopsy. Furthermore, immunofluoresence techniques are used to detect *in vivo* depositions of IgG autoantibodies in the epidermis and anti-epithelial intercellular substance (ICS) IgG circulating in the blood.

Presentation

Pemphigus has a world-wide distribution with an incidence of approximately 0.1-0.42 per 100.000 per year (1-3). Pemphigus is a rare disease in Europe and North America. The prevalence and incidence of pemphigus in patients of Jewish origin is increased (1,6-3,2 per 100.000) and endemic regions are known in Tunisia, Iran and India (1;4).

The disease has a peak incidence of occurrence in patients between the fourth and sixth decade. About 0.8% of all dermatological patients suffer from pemphigus (4). For the Netherlands, the total year incidence of pemphigus is assessed to be about 46 casus (0,29 per 100.000), based on the counted number 177 patients who had a skin biopsy taken with the diagnosis of pemphigus examined by Dutch pathologists over the years 1995 and 1996 (unpublished data derived from the Dutch PALGA database).

Classification

The pemphigus spectrum is classified based on 1) clinico-pathological presentation, 2) type of autoantigens, and 3) subclass of autoantibodies. Table I and II show the pemphigus subtypes based on subclass of autoantibody (IgG or IgA) in combination with the targeted autoantigen.

The two main categories of pemphigus are pemphigus vulgaris (PV, vulgaris = ordinary) and pemphigus foliaceus (PF, folia = leaves). Pemphigus vulgaris (PV) is the most common type of pemphigus and comprises about 80% of patients with pemphigus (5). In about 50-70% (4;6) of the cases the disease begins with oral lesions, which may precede the cutaneous lesions by several months or be the major, if not only, manifestation in some patients (4). The mucous membranes are ultimately involved in most cases of PV. The histology of PV shows acantholysis in the lower epidermis with suprabasal blister formation.

Pemphigus foliaceus (PF) comprises about 20% of the patients with pemphigus. In PF only skin is affected, the mucous membranes are never involved (7), The allege that mucous membranes are always unaffected in PF is disputed by others (8). The histological level of blistering in PF is more superficial than in PV at the level above, in, or just beneath the granular layer (9). PF emerges with crusted squamous plaques in seborrhoic areas, and therefore may be mistaken for seborrhoic dermatitis, severe actinic keratosis, lupus erythematosus, psoriasis, or impetigo. However, PF may initially also erupt with generalized flaccid blisters. According to our experience the split level may then be observed in the mid-epidermis, 2-3 layers beneath the stratum granulosum. PF with flaccid blisters is differentiated from PV by the lack of mucous membrane involvement and the absence of anti-desmoglein 3 antibodies.

Pemphigus vegetans, a subtype of PV, has been divided classically in two subtypes, Neumann and Hallopeau (10). In the Neumann-type so-called vegetations (papillomatous granulations) sometimes hemmed with peripheral pustules develop on denuded areas surrounding orificiae. In the Hallopeau-type pustules are more common, they are rapidly followed by vegetations in often affected intertriginous areas. Also in PV vegatations on the face may develop, clinically and histopathologically characterized by papillomatosis. It is probably accurate to think of pemphigus vegetans as a clinical variant of PV.

Table I Classification of pemphigus mediated by IgG [Jonkman, abstract EADV 2001]

Disease subtype	*Antigen*
Pemphigus vulgaris	desmoglein 3, (desmoglein 1), pemphaxin?
Pemphigus vegetans, Hallopeau type	desmoglein 3, (desmoglein 1)
Pemphigus vegetans, Neumann type	desmoglein 3, (desmoglein 1)
Neonatal pemphigus vulgaris	desmoglein 3, (desmoglein 1)
Paraneoplastic pemphigus / Paraneoplastic autoimmune multiorgan syndrome (PAMS)	desmoglein 3, (desmoglein 1) + plectin, desmoplakin I/ II, 230-kDa bullous pemphigoid antigen, envoplakin, periplakin, 170-kDa antigen
Drug-induced pemphigus	desmoglein 1, (desmoglein 3)
Pemphigus foliaceus	desmoglein 1
Pemphigus herpetiformis	desmoglein 1, (desmoglein 3)
Pemphigus erythematodes	desmoglein 1
Neonatal pemphigus foliaceus	desmoglein 1
Fogo selvagem	desmoglein 1

Pemphigus herpetiformis is an unusual pruritic variant of pemphigus vulgaris. Clinically it resembles dermatitis herpetiformis, whereas histopathological and imunofluorescence examination yield findings diagnostic for pemphigus (11;12).

Another unusual manifestation of pemphigus is neonatal pemphigus. Transplacental passage of IgG during pregnancy in affected patients is thought to be the course of PV or PF (13,14).

The paraneoplastic subtype of pemphigus (PNP) is often associated with unusual lymphoreticular malignancies (15). Diagnostic for PNP is the presence of circulating autoantibodies against plakins (plectin, desmoplakin I/ II, 230-kDa bullous pemphigoid

antigen, envoplakin, periplakin, and an uncharacterized 170-kDa antigen) which are detectable by immunoprecipitation or immunoblot (15). It is suggested that paraneoplastic pemphigus (PNP) is a heterogeneous autoimmune syndrome involving several internal organs: paraneoplastic autoimmune multiorgan syndrome (PAMS) (16).

In the skin a spectrum of at least 5 different clinical and immunopathological mucocutaneous variants are noted (i.e. pemphigus-like, pemphigoid-like, erythema multiforme-like, graft-vs-host disease-like, and lichen planus-like). The pathophysiological mechanisms of PAMS involve both humoral and cellular autoimmunity responses. Epithelial cell membrane antigens other than Dsg1 or Dsg3 are targeted by effectors of PAMS autoimmunity. Apoptosis of damaged basal cells mediates epithelial clefting, and respiratory failure results possibly from obstruction of small airways with sloughed epithelial cells.

Drug-induced pemphigus was first described in 1969 by Degos in patients using penicillamin (17). It is demonstrated that autoantibodies from drug-induced pemphigus patients have the same antigenic specificity, on a molecular level, as autoantibodies from other pemphigus patients (18,19). The chance of acquiring pemphigus after penicillinamin intake of least 6 months is 7% (20). Since then, more medications were reported to evoke pemphigus, such as penicillin, ampicillin, rifampicin, pyrazolon derivates, a combination of aspirin and indomethacin, and a combination of propanolol and mepbromate (21). Drugs ‘at risk’ for pemphigus are sulfhydryl (SH)-group containing drugs, known as thiol-drugs (i.e. captopril) (22). Drug-induced and drug-triggered pemphigus are considered to be separate entities (22). In case of *drug-induced,* exogenous, non-autoimmune factors play a major role, and the disease regresses when the offended drug is discontinued (23). In *drug-triggered* pemphigus, the drug only stimulates a predisposition (endogenous and genetic factors) to develop active autoimmune disease. It seems that penicillamin and SH-containing drugs actually induce pemphigus, whereas other drugs only trigger a disimmune mechanism previously programmed and ready to be set off (22). Drug-triggered pemphigus is known to be refractory to therapy if the offending drug is not stopped immediately. Dietary factors, containing chemical compound resembling the above mentioned drugs, such as thiols

(garlic, onion, celery), isothiocyanates (mustard, horseradish), phenols (mango, cashew), and tannins (cassava, red chillies, tea, red wine) are also mentioned as exogenous factors to trigger pemphigus in genetically predisposed persons (24).

Pemphigus erythematosus (Senear-Usher), a subset of PF, usually presents with concomitant deposition of immunoglobulins and complement along the epidermal basement membrane zone in lesional skin in addition to pemphigus staining pattern in the epidermis (25).

Endemic pemphigus foliaceus, fogo selvagem, wildfire, or Brazilian PF, occurs predominantly in central and southern Brazil. In contrast to PF, fogo selvagem occurs in endemic foci, and often affects children and young adults (26). The etiology of fogo selvagem is still unknown. The frequent association with insect bites has lead to the concept of fogo selvagem being a transmissible disease with acquired immunity in adulthood. However, the infectious agent and possible vectors have not yet been identified (27).

Table II Classification of pemphigus mediated by IgA only

Disease subtype	*Antigen*
Subcorneal pustular dermatosis	Desmocollin 1
Intraepidermal neutrophilic IgA dermatosis	Desmoglein 3?
IgA-pemphigus vulgaris	Desmoglein 3 (Desmoglein 1)
IgA-pemphigus foliaceus	Desmoglein 1

Recently IgA-pemphigus was identified, characterized by immunoglobulin A (IgA)-type autoantibodies directed against keratinocyte cell surfaces (28). There is now evidence that IgA pemphigus encompasses at least two subgroups: a subcorneal pustular dermatosis (SPD)-type, characterized by subcorneal pustules and autoantibodies to desmocollin 1; and the intra-epidermal neutrophilic dermatosis (IEN)-type cases which show intraepidermal pustules and in whom the autoantigen is variable but may be desmoglein 3, the pemphigus vulgaris antigen (29). The treatment of IgA-mediated pemphigus is different from that of IgG-mediated pemphigus.

Transformation between the PV and PF categories is possible, although rare. Nevertheless if such transformation does occur, then a shift from PV to PF is more common (30-36) than, vice versa, a shift from PF to PV (33;36;37). The disease period before transition may vary between 1-20 years (30). In chapter 7 a patient is reported transforming from PV to PF.

Pathogenesis

The molecular pathomechanism disregulating keratinocyte adhesion in pemphigus are to a far extend elucidated. The unraveled pathogenesis makes pemphigus a model of organ-specic humoral autoimmune diseases. Figure I depicts the desmosomal complex, which plays the major role in the cell-cell adhesion of keratinocytes (38). Despite the solid evidence that anti-desmoglein (Dsg) antibodies play a central role in the pathofysiology of PV and PF (39;40), the significance of non-desmoglein antibodies in the pathogenesis is raised in recent reports (41;42).

Stanley and Udey formulated the *desmoglein compensation hypothesis*, which explains the differences in skin and mucous membranes involvement between PV and PF (40). Patients with PF have antibodies exclusively reactive with Dsg1. Patients with PV have antibodies reactive to Dsg3, but may also have antibodies to Dsg1. It has been demonstrated that early in the course of PV, when lesions are limited to mucous membranes, patients tend to have antibodies only against Dsg3. Furthermore, it is not inevitable that all patients with Dsg 3 antibodies will ultimately develop Dsg 1 antibodies. Anti-Dsg1 antibodies originate later in the disease progress of PV, coinciding with skin involvement (40). In figure II the triangles represent the distribution of Dsg1 and Dsg3 in adult skin and mucous membranes. In PF the patients' serum contains only anti-Dsg1 antibodies and cause blisters by interfering with the function of Dsg1 in the upper epidermis, where there is no Dsg3 to compensate. In early pemphigus vulgaris, when patients produce only anti-Dsg3 antibodies, suprabasal blisters develop only in mucous membranes, where Dsg 1 cannot compensate for the loss of Dsg3 mediated adhesion. Later in the course of PV, when the patients' sera contains anti-Dsg1 and anti-Dsg3 antibodies, the function of both Dsgs is compromised and blisters occur also on the skin. The desmoglein compensation hypothesis does not cover the observation that

patients with Dsg 3 antibodies alone can get mild blistering of the skin (patient 4, chapter V). The validity of the compensation theory has been confirmed in experiments with PF and PV antibodies in normal and Dsg3 null mice (44). This study suggest that pemphigus autoantibodies inhibit the adhesive function of desmoglein proteins, and demonstrates that either Dsg1 or Dsg3 alone is sufficient to maintain keratinocyte adhesion.

The multiple hit hypothesis is another hypothesis to explain acantholysis in pemphigus and fosters development of non-steroidal treatments. Both Dsg and non-Dsg autoantibodies are required to induce blisters. As recently reviewed (45), a list of known autoantigens in pemphigus includes both adhesion molecules (Dsg1, Dsg2, and Dsg3, desmocollins, plakoglobin, collagen XVII/BP180) and receptor molecules (α3 AchR, α9 AchR, pemphaxin and other annexins) (46).

Data supporting a role for non-Dsg autoantigen role in the pathogenesis of pemphigus include the following (47):

1. PV sera devoid of anti-Dsg1 activity produce new blisters in Dsg3 (-/-) mice. Dsg3 (-/-) mice develop spontaneous few blisters, but after injection of PV sera develop massive new blistering, similar to that in Dsg3 (+/+) mice. This confirms the importance of inactivating Dsg3 to produce PV-like blisters, but also indicates that anti-Dsg antibodies are not the sole pathogenic antibodies in PV sera.
2. PV sera contain multiple non-Dsg antigens from both normal, and Dsg3 (-/-) keratinocytes, including an antigen (not Dsg3) that migrates at 130 kDa.
3. Absorption of PV sera with Dsg3-Ig fusion protein removes pathogenicity; however, antibodies eluted from the Dsg3-column react with multiple antigens from both Dsg3 (-/-) and normal keratinocytes (47).
4. From 34 to 71% of first relatives of PV patients have anti-Dsg1 or anti-Dsg3 antibodies without any clinical signs of pemphigus (47;48).

The above data suggest that PV is also mediated by non-Dsg autoantibodies acting in concert with those against Dsg. One of the non-Dsg antigens is the keratinocyte α9 acetylcholine receptor (47) and a 130 kDa antigen pemphaxin (45). This notion may open a novel approach for the treatment of pemphigus using cholinergic agonists (pyridostigmine or Mestinon®) (49,50).

The desmoglein compensation theory is incompatible with the "older" protease (plasmin) theory of blister formation. This theory suggests that after binding of autoantibodies production of urokinase type plasminogen activator is stimulated (51). Plasminogen is converted to plasmin, which may be responsible for loss in strength of intra- or extracellular components of the desmosomal complex, resulting in acantholysis (52). Mahoney et al. demonstrated however that plasminogen activator is not necessary for pemphigus immunoglobulin to induce acantholysis in the neonatal mouse model of pemphigus (53). Caldelari et al (54) recently demonstrated in an *in vitro* model that the intracellular component, plakoglobin, plays a major role in the pathogenesis of pemphigus. Binding of PV IgG to plakoglobin knockout keratinocytes did not induce acantholysis. When full-length plakoglobin was reintroduced into the plakoglobin knockout cells, responsiveness to PV IgG was restored. This study excludes the steric hindrance-only-hypothesis of IgG binding to the extracellular portion of the desmosome.

Therapy

'How to treat pemphigus?', has always been a difficult question, since the disease may break through milder treatment modalities, whereas aggressive immunosuppressive drugs require careful monitoring of the patient. Choice of treatment, i.e. first and second line immunomodulators, depends on disease phase. For instance, in mild orally affected patients, topical or intralesional corticosteroids or tetracycline mouthwashes may be started as first line therapy. Whereas the severe affected patient needs to be treated with systemic high dose immunosuppressive therapy.

In the Cochrane Controlled Trials Register and PubMed database are no systematic reviews (meta-analysis), or large randomized clinical trials (RCT) available for treatment of pemphigus. There are only 4 RCT's with a Sackett level of evidence of at least II (55).

Sackett levels of evidence and clinical recommendation

Grade of recommendation	*Level of evidence*	
A	Ia	metananalysis of RCT's
A	Ib	large RCT with clear cut results and low risk of error
B	II	small RCT with uncertain results and moderate to high risk of error
B	III	non-randomized contemporaneous controls
C	IV	historical controls
C	V	no controls, case series
D	VI	expert opinion, and case report

Since their introduction in the 1950s of the previous century, the cornerstone of therapy remains corticosteroid treatment at minimal effective dosage.

To minimalise iatrogenic effects of cumulative corticosteroids, there has been a continuous search for alternative therapies regarding treatment during the last 40 years (56-58). These include azathioprine, cyclophosphamide, methotrexate, mycophenolate mofetil, pulse glucocorticoid therapy, gold, tetracyclines, dapson, intravenous immunoglobines, plasma exchange, immunoapheresis, and intralesional steroids. Because these modalities are normally used in conjunction with oral daily systemic steroids, rather than as monotherapy, these modalities can be called adjuvant therapies.

Adjuvant therapy is started immediately with the glucocorticoid treatment, or after an initial period of treatment with oral glucocorticoids only. Reasons for adding adjuvant in a later stage are:

- unsatisfactory initial clinical effect of oral steroids,
- sustained need for high dose maintenance steroid therapy,
- presence of steroid related adverse events,
- intention to reduce steroid related adverse events.

Chapter II summarizes current therapy in pemphigus.

Prognosis

Without therapy, pemphigus is fatal within the first year of onset in 75% of the patients due to sepsis and loss of body fluids (59;60). Since the use of steroids in 1950,

pemphigus has transformed from an almost invariably fatal disease into one whose mortality is now about 5% (61;62). Mortality at present is determined by the complications of the therapy. Evaluation of three recent studies show that sepsis and lung embolism are the main causes of death, caused by the steroid treatment (59;62;63).

Morbidity of pemphigus did not further decrease since the introduction of steroids, attributed to the iatrogenic effects of the therapy (57;59;63-65). The iatrogenic morbidity is responsible for the largest part of costs that these patients generate. Therapy can be discontinued in approximately 75% of all patients after 10 years (61). About 25% of the patients remain dependant of both steroids and adjuvant treatment during life.

The most relevant adverse events are steroid-induced diabetes mellitus, hypertension, infection (cave masking of symptoms), ulcus pepticum (bleeding, perforation, cave masking), increased clotting tendency, osteoporosis, and delayed wound healing. Furthermore, adverse events are provoked by the scala of adjuvant therapies (66).

The induction of complete remission was studied in a long-term longitudinal study in 40 patients with pemphigus vulgaris treated conventionally and followed up for an average of 7.7 years by the same investigator (61).The course of the disease follows different patterns, with respectively 25%, 50%, and 75% of the patients reaching complete remission 2, 5, and 10 years after the diagnosis pemphigus. Induction of complete remission is related to initial severity, extend of disease, and early response to treatment. The rate of remissions in pemphigus is unclear because these are usually reported at a single point in the evolution of the disease. Thus it is uncertain whether treatment simply suppresses the manifestations of the disease and consequently must be continuously administered, or induces complete and long-lasting remissions that permit therapy to be discontinued.

Pulse therapy

Pulse therapy, the ‘big shot’ (67), refers to discontinuous intravenous infusion of very high doses glucocorticoids over a short time. Doses of each pulse are not standardised, but are usually 500-1000 mg methylprednisolone or 100-200 mg dexamethasone. The aim of pulse therapy is getting quicker and stronger efficacy and decreasing the need for

long-term use of steroids. The contradiction is that pulsed administration of high dose steroids is used to achieve the steroid-sparing effect.

Pulse therapy was initially proposed for the emergency treatment of acute rejection of kidney transplants 30 years ago (67), and it is still first choice for the treatment of acute rejection. Now, it is also widely used in the treatment of many inflammatory or autoimmune diseases.

The largest experience with glucocorticoid pulse therapy in dermatology is obtained in patients with pemphigus vulgaris. Pasricha *et al.* described both a steroid-sparing effect, 84% complete remission rate, and long-term remission of up to 9 years, using pulse therapy (68). We retrospectively studied the sequelae of 14 patients with pemphigus who were treated by pulse therapy (chapter 4).

Pulse therapy is mostly given intravenously rather than orally, without evidence to support the necessity of the intravenous route. Oral pulse therapy is preferable, since it avoids vena puncture, reduces costs, and is more convenient for the patient. An oral formulation for dexamethasone pulse therapy was also necessary for blinding the clinical trial by placebo, in which the therapeutic effect of corticosteroid pulses will be evaluated. Intravenous placebo pulses may be considered unethical. Dexamethasone was chosen for use in oral pulses. To develop a suitable dosage for oral pulses, bioavailability of high-dose dexamethasone had to be determined. Chapter 3 explains the pharmacokinetics of high-dose oral dexamethasone pulse therapy.

Glucocorticosteroids

Glucocorticosteroids are important anti-inflammatory and immunosupressive drugs with three distinct mechanisms of action: 1) genomic, 2) specific non-genomic, and 3) unspecific non-genomic. The (classical) genomic action mechanism is glucocorticoid receptor mediated. Glucocorticoids bind to the cytosolic expressed glucocorticoid receptor. After binding the activated steroid-receptor complex translocates to the nucleus, where synthesis of regulating proteins, e.g. lipocortin-1, an inhibitor of phospholipase A2, is initiated. The steroid receptor complex also interacts with transcription factors, modulating transcription of messenger RNA and subsequent decreased synthesis of certain proinflammatory cytokines and increased synthesis of anti-inflammatory

cytokines. These genomic actions are observed at any corticosteroid dose, and occur later than 30 minutes after binding of the glucocorticoid at the receptor. Additional non-genomic effects have been shown (*in vitro*) to occur only at high-doses, above 250 mg prednisolone equivalent per day (69-71).

Specific non-genomic effects occur rapidly (seconds or minutes) and result mainly from direct interaction on biologic cell membranes and are supposed to interfere with activation and maintenance of immune cells. In therapeutically relevant concentrations, methylprednisolone instantaneously inhibits Ca^{2+} and Na^{+} ions cycling across the membranes and decreases intracellular free Ca^{2+} concentration, but has little effect on protein synthesis (71). Further non-genomic effects are a decreased phospholipid turnover in the cell membranes and a decreased production of free radicals (72).

Pulse therapy of high-dose glucocorticoids thus may have additional unspecific non-genomic effects, and therefore may lead to rapid and intense immune responses. This was demonstrated in a recent study in children with autoimmune diseases where additional non-genomic effects were observed due to pulse therapy despite a significant down-regulation of glucocorticoid receptors in these children (69).

How does puls therapy work in pemphigus is uncertain. Corticosteroid pulses reduce skin blistering within days, when autoantibody titres are not yet lowered. Recently, Stanley hypothesized that high dose corticosteroids pulses might induce transcription of Dsg isoforms, providing protection from anti-Dsg autoantibodies (76). Alternatively, we speculate that corticosteroids may strengthen desmosomes by modulating intracellular calcium oscillations, which for instance may revert plakoglobin-dependant acantholysis.

Clinical and laboratory monitoring

At present, no consensus is available for clinical scoring disease activity, and therefore monitoring pemphigus. There is a need for a uniform simple scoring system with a small interobserver bias, since pemphigus is a rare disease.

Both for diagnosis and follow-up immunological techniques are used to detect specific *in vivo* depositions of IgG in the epidermis and circulating IgG in blood. In 1964, Beutner and Jordon (73) first demonstrated antibodies to the cell surface of epidermal cells in the sera of patients suffering from PV. Since then indirect IF is used widely for

monitoring disease activity, however pemphigus antibody titres do not always correlate with actual disease activity (74). Classically, immunoprecipitation is used to identify pemphigus antigens in a research setting. The technique is available in few centers worldwide. PV is characterized by antibodies against the 130 kDa PV antigen (desmoglein 3), and PF by antibodies against the 160 kDa PF antigen (desmoglein 1). Alternatively, immunoblot may be used, but this technique is often false-negative for anti-desmoglein antibody detection. The usefulness of immunoblotting in pemphigus diagnostics is mainly restricted to identifying paraneoplastic pemphigus, where an highly specific plakin pattern can be found, and to IgA-pemphigus were IgA against desmocollins and desmogleins can be detected (15). The breakthrough in pemphigus diagnostics came in 1997, with the development of an ELISA (enzyme-linked immunosorbant assay (MBL, Nagoya, Japan) to detect specific autoantibodies against the ectodomains of desmoglein 1 and 3 (43). The technique is highly sensitive and specific, and also quantitative. Monitoring pemphigus by quantifying circulating anti-desmoglein antibodies with ELISA therefore seems to be an attractive option.

Chapter 5 proposes a model for robust staging of disease activity for pemphigus vulgaris. Uniform monitoring pemphigus vulgaris, with a small interobserver bias, due to a simple clinical definition seems now possible. This clinical definition is currently used in our PEMPULS trial, an international, prospective, multi-centre, double-blind, placebo-controlled, parallel-group, randomized clinical trial, in which the efficacy of oral high-dose dexamethasone pulse therapy is studied. Participating countries are: the Netherlands, Belgium, France, Germany, Hungary, Italy, Poland, Spain, and the United Kingdom. All information for participants and others who are interested is public available on our website http://www.pempuls.nl.

Safety of High-dose Azathioprine

Standard therapy in the Netherlands for pemphigus comprises maintenance dosage prednisolone in combination with azathioprine. Myelotoxicity and drug efficacy are now known to be related to the activity of the key enzyme in azathioprine metabolism, thiopurine methyltransferase (TPMT). The drug has well-documented toxic effects on hematopoietic cells that may be acute or chronic, including: macrocytosis, anemia,

trombocytopenia, leukopenia, pancytopenia, and acute bone marrow failure. About 1 of 300 patients are homozygous for the inactive TPMT allele (75). Azathioprine should not be prescribed to such patients that will otherwise develop acute myelosuppression. TPMT as measured in erythrocytes also discloses patients with an high TPMT activity. On standard dosage of 100-150 mg azathioprine per day, patients with a high azathioprine metabolism would be suboptimal treated, and should receive more azathioprine.

We reviewed the sequelae of 14 dermatological patients on high-dose azathioprine, and we assessed the clinical value and suitability of the TPMT assay in clinical practice in this small group of patients who need sufficient immunosuppression (chapter 6).

Aims of this study

The groundwork for this thesis started in May, 1997 with the design of the PEMPULS trial that was initially applied to the Dutch 'Ontwikkelingsgeneeskunde' program. Since treatment of pemphigus is not an economic issue in public health care, the proposal was rejected. Pemphigus is an orphan disease. Dexamethasone is an orphan drug lacking patent protection so that no pharmaceutical industry was interested either. With limited financial support of the Faculty of Medical Sciences of the University of Groningen and the University Hospital Groningen we embarked for an international, prospective, multi-centre, double-blind, placebo-controlled, parallel-group, randomized clinical trial, in which the efficacy of oral high-dose dexamethason pulse therapy is studied. The academy driven trial is carried out by the network of the European Society of Autoimmune Bullous Diseases on a non-profit basis. The scientific question whether pulse therapy really works as adjuvans in pemphigus apparently raised the enthusiasm of the participants. First, the results of dexamethasone pulse therapy in our department were studied retrospectively in a pilot-study (Chapter 4). A literature search was performed to evaluate all therapies in pemphigus (Chapter 2). An attempt was made to design definitions for monitoring disease activity (Chapter 5). To design the oral substitute (necessary for placebo control) for intravenous dexamethasone pulse therapy, we started pharmacokinetic studies on our ward (Chapter 3). Since high-dose azathioprine is used as first choice adjuvant in the Netherlands, we started measuring TPMT-enzyme activity to avoid serious adverse events (Chapter 6).

Fig I. Transmembrane desmosomal adhesionproteins of the desmosomal complex.

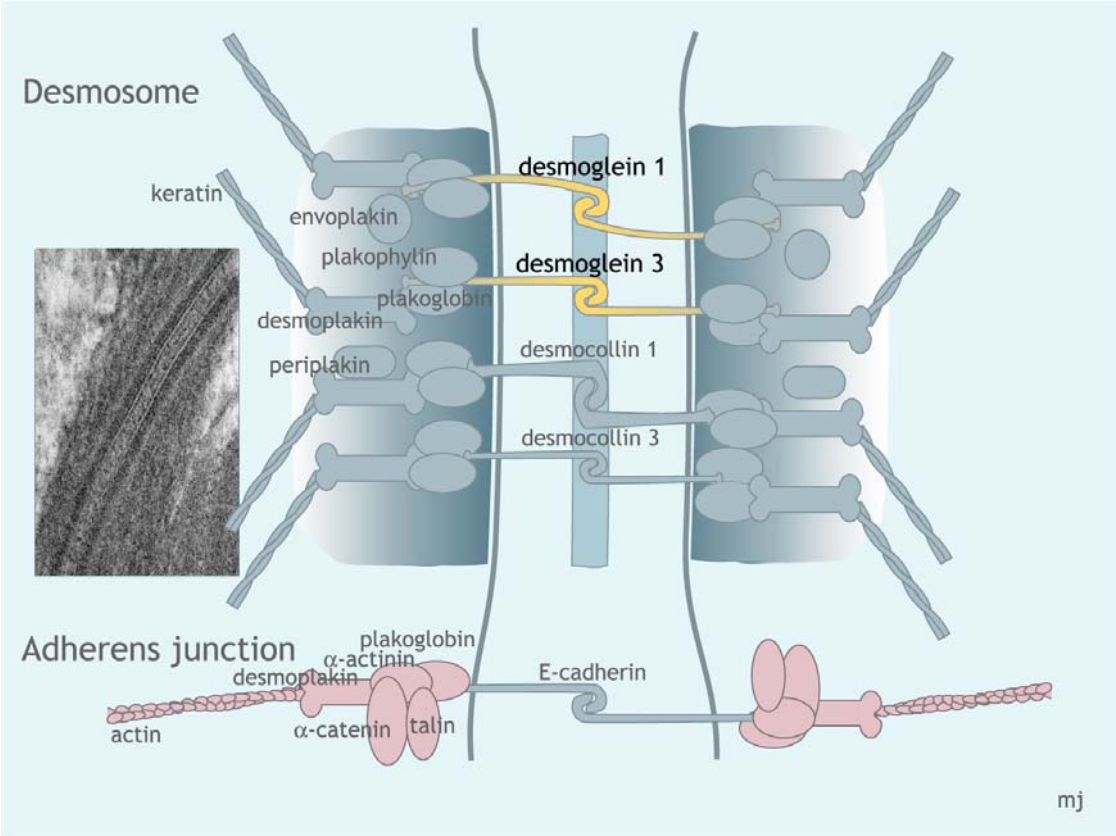
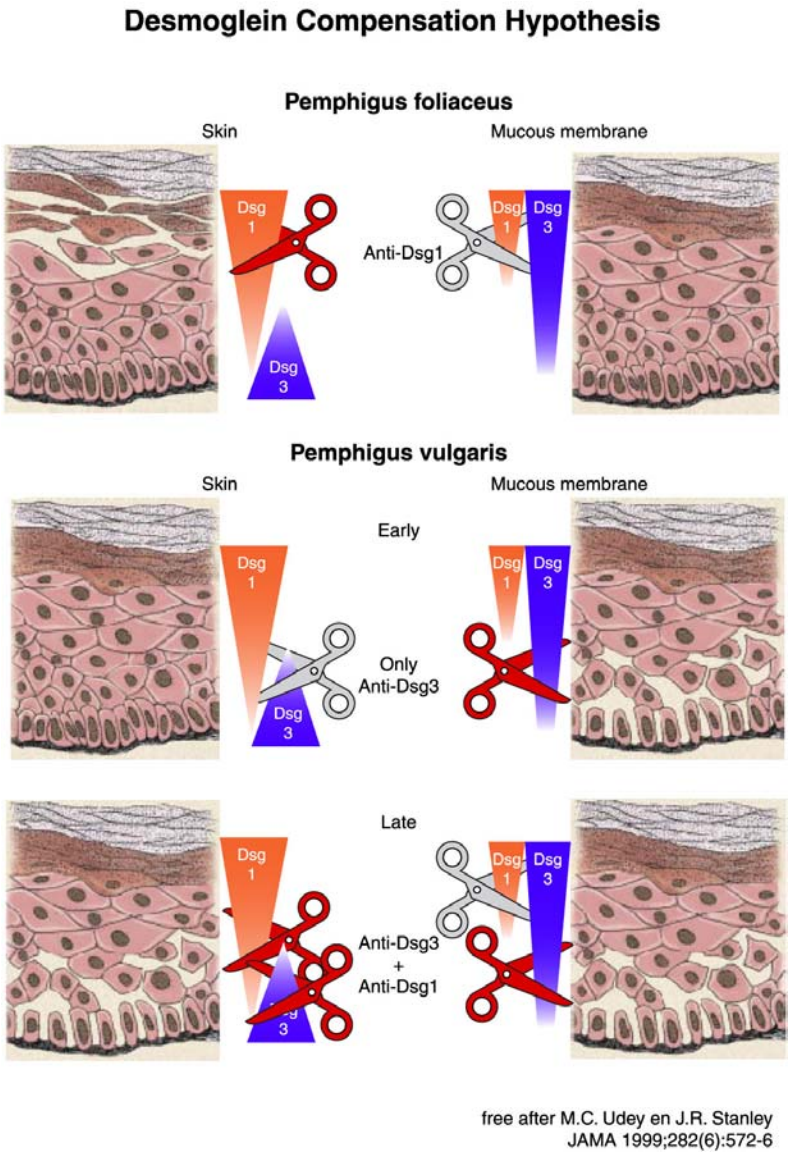


Fig II. The desmoglein compensation theory. The triangles represent the distribution of Dsg1 and Dsg3 in adult skin and mucous membranes.



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Chapter II

Therapy of pemphigus

Abstract

Systemic glucocorticoids are still the cornerstone in treatment of pemphigus, but constitute a considerable health risk when used long-term. New treatment strategies focus first on averting the use of systemic glucocorticoids by using topical glucocorticoids, nicotinamide and tetracyclins as initial treatment in milder cases. The second focus is on reducing the daily glucocorticoid dose by using steroid-sparing adjuvans, such as azathioprine, mycophenolate mofetil, glucocorticoids pulse therapy, methotrexate, and intravenous immunoglobulins. The aim is to lower the morbidity caused by the treatment of this autoimmune disease, which often follows a chronic course.

Tóth GG, Jonkman MF. Therapy of pemphigus. Clin Dermatol 2001;19:761-7.

Introduction

Pemphigus is a severe chronic autoimmune blistering disease of skin and mucous membranes which was lethal until 1950s when glucocorticoids became available. Glucocorticoids are the cornerstone of treatment. However, the disease is still difficult to treat, and the long-term use of steroids leads to severe side-effects. Pemphigus is a rare disease with an annual incidence of approximately 0.1-0.42 per 100.000 (1-3). A peak incidence occurs in patients between the fourth and sixth decade. About 0.8% of all dermatological patients suffer from pemphigus (4). Pemphigus vulgaris (PV) is the most common subtype and comprises about 80% of patients with pemphigus (5). In about 50-70% (4;6) of the PV cases the disease begins with oral lesions, which may precede the cutaneous lesions by several months or be the major, if not only (4), manifestation in some patients. The mucous membranes are ultimately involved in most cases of PV.

In about 20% of the pemphigus patients, the subtype is pemphigus foliaceus (PF). In PF only skin is affected (7), and its course is less severe than in PV. PF patients are often treated with milder treatment modalities, although this subtype appears to be very chronic and sometimes refractory to therapy.

'How to treat pemphigus?', has always been a difficult question, since the disease may break through milder treatment modalities, whereas robust immunosuppressive modalities require careful monitoring of the patient. Choice of treatment, i.e. first and second line immunomodulators, depends on disease phase. For instance, in mild orally affected patients, glucocorticoid- or tetracycline mouthwashes can be started as first line therapy. Whereas the severe affected patient needs to be treated with robust immuno-suppressive modalities. Bystryn and Steinman reported in reviews of pemphigus therapy that the available literature has to be interpreted carefully for several methodological flaws in the research approaches, which are inseparable linked to the low incidence of this bullous autoimmune disease (8;9).

ELISA is a new diagnostic tool that measures circulating autoantibodies against desmoglein 1 and 3. A positive correlation between ELISA values and IIF titers was found in 11 PV patients by Lenz et al. (10). Aoyama et al. suggested to use the ELISA titer for desmoglein 1 for determining the initial therapy for PF (11). PF patients with low

ELISA titre were treated with topical steroids, whereas those with high titres with high-dose glucocorticoid pulse therapy.

Topical glucocorticoids

Topical application of potent steroids can be considered in mild cases of pemphigus. Topical steroid therapy alone was considered to be insufficient for sustained disease control, since pemphigus is a systemic autoimmune disease, and as long as there are adequate amounts of circulating autoantibodies binding to the skin, lesions will continue to develop. Recent uncontrolled studies however report successful treatment of mild cases with bullous pemphigoid (12), and pemphigus (13). Clobetasol propionate 0.05% cream was applied twice daily on pemphigus lesions for 15 days. Skin lesions seemed to be controlled in all patients within this period. For mucosal lesions, in pemphigus vulgaris, it took at least one month. However, a trend to relapse, mostly in previous affected areas, was observed during tapering of the topical treatment. In our experience intralesional triamcinolon may be used successfully in cases with solitary erosions in the mouth or with localised pemphigus.

Systemic glucocorticoids

As mentioned before, systemic glucocorticoids are a major breakthrough in the treatment of pemphigus (9;14). Glucocorticoids transformed pemphigus from an almost invariably fatal disease into one whose mortality is now between 5 to 10% (9;15;16). At present, most patients who die of pemphigus do so through complications of therapy, especially when long-term daily glucocorticoids at high-dose are needed to sustain disease-control. Minimising the incidence and severity of glucocorticoid-related side effects requires carefully decreasing the dose; using disease-modifying immunosuppressive and anti-inflammatory agents as adjuvans; and taking general preventive measures (8;9).

At the molecular level, glucocorticoids form complexes with their specific glucocorticoid-receptors that migrate to the nucleus where they interact with selective regulatory sites on the DNA; this results in direct positive and negative modulation of several genes critical in inflammatory and immune responses (17). A second molecular

mechanism of glucocorticoids was recently shown to induce the synthesis of IκB, thus antagonising NF-κB translocation to the nucleus and gene transcription (18;19). A prompt inhibition of the inflammatory response is effected, but also other cells like fibroblasts and keratinocytes are inhibited.

A recent study showed that in approximately 75% of the patients after 10 years systemic therapy can be safely discontinued without a flare in disease activity (20). Morbidity in pemphigus is mostly iatrogenic, especially when long-term high-dose steroids are needed to sustain disease control (9;21-23). Most relevant adverse effects of glucocorticoids are diabetes mellitus, hypertension, infection (cave masking of diverticulosis), ulcus pepticum (bleeding, perforation, cave masking), increased clotting tendency, osteoporosis, acne, and delayed wound healing (21).

Prednisolone is the preferable glucocorticoid for oral administration, since it is the active hydroxylated metabolite of prednisone after liver passage. We use the ester form of prednisolone, since the salt-formulation of prednisolone, prednisolone-metasulfbenzoate, is even less absorbed than the prodrug prednisone (24;25). If the prednisolone-ester is not available like in France, prednisone is advised (24). There is no consensus regarding the initial steroid dosage needed to induce remission and the effect of this on the subsequent course of the disease. The controlled trial of Ratnam et al. (26) demonstrated that moderate dose of prednisone, 60 mg/day was effective in controlling pemphigus.

In our experience low doses (below 60 mg / day) usually do not suffice to induce initial control, especially when skin is affected. We use 80 mg/day as starting dose in a clinical setting. If no initial control after one week, daily dose is increased according to the Lever regimen (27). This means that in case of a severe pemphigus daily dosage of 240 mg can be reached in two weeks to prevent new blister formation (table I). After initial control prednisolone is tapered weekly until 40 mg/day is reached. Subsequently the dose is tapered in 4 months until zero. In case of new blister formation, i.e. positive disease activity, go two steps back in the tapering schedule (table II), and keep for two weeks. It is also possible to taper from 10 mg on in 1-mg steps (use 1-mg prednisolone tablets). Most pemphigus patients do need maintenance schedule prednisolone varying

from about 5-15 mg/day. Alternating daily doses for administration of steroids are well-know to cause less side-effects on the hypothalamic-pituitary-adrenal axis function (68). However on non-steroid days the patients may complain of a sensation of erythema, or itch. Standard we prescribe a H_2-blocker, ranitidine 150 mg, to prevent steroid-induced dyspepsia.

Table I: Oral prednisone schedule for initial control

Week	*Prednisolone (mg/day) schedule*		
1	80	80	80
2	60	120	120
3	40	60	240
4		40	120
5			60
6			40

Table II: Oral prednisone schedule for tapering of maintenance dose after tapering schedule table 1

Step	*Period (number of weeks)*	*Daily schedule Prednisolone (mg/day)*
7	2	30
8	2	20
9	2	15
10	2	12,5
11	2	10
12	2	7,5
13	2	5
14	2	2,5
15	-	0

In case of gastric complaints we increase the ranitidine to 150 mg bd. or prescribe a protonpump-inhibitor, such as omeprazol 20 mg dd.. To prevent secondary osteoporosis and (spine) fractures calcium 1000 mg dd. is advised as co-medication. Daily prednisolone dosage of 7,5 mg or more is an indication for bone densitometry. In case of low bone density (T- or Z-score $\leq$ -1), or in postmenopausal females bifosphonate is added (400 mg etidroinacid or 10 mg alendroinacid during 14 days every 3 months)

(28). Alternatively, you may recommend drinking 6 glasses milk per day and ad vitamin D, 400-800 IE colecalciferol per day, to the medication list. Further precautions are monitoring of blood pressure, and blood sugar. For patients having symptoms suggestive for nasal/pharyngeal involvement or ophtalmological side-effects, specialists are consulted. An adequate medical history should always detect suppurative infections in the immunosuppressed patient.

High-dose glucocorticoid pulse therapy

Pulse therapy, the 'big shot' (29), refers to discontinuous intravenous infusion of very high doses glucocorticoids over a short time. Pulse therapy is used as adjuvant in combination with maintenance schedule prednisolone and azathioprine, since reviewing the published cases no increased efficacy has been found with pulse glucocorticoid therapy alone (29). However in our experience, monthly glucocorticoid pulses are effective as monotherapy in patients with mild oral lesions in the early stage of pemphigus vulgaris. Noteworthy, in our experience pulse therapy alone does not induce Cushingoid side-effects, whereas even daily low-doses of prednisolone induce the Cushingoid habitus within 8 weeks. Contra-indications for pulse therapy are chronic or recurrent infections (diverticulitis, herpes simplex oculi), tuberculosis, cardial dysrhytmias (atrial fibrillation is no contra-indication), low serum potassium, morbus Cushing , and pregnancy. Be aware of psychosis or lability in patients with psychiatric medical history.

Doses of each pulse are not standardised, but are usually 500-1000 mg methylprednisolone or 100-200 mg dexamethasone. We have chosen dexamethasone, a fluoridated glucocorticoid, for pulse therapy. Dexamethasone is per mg about 6.7 stronger than prednisolone on the hypothalamic axis, the mineralocorticoid effect is neglectible, and is has a very low equipotent volume (21). In the literature a variation in choice of steroid, dosage of pulse and cycle repeat are mentioned. The aim of pulse therapy is getting quicker and stronger efficacy of glucocorticoid therapy and decreasing the need for long-term use of steroids. The paradox is that pulsed administration of high dose steroids is used to achieve the steroid-sparing effect.

The largest experience with pulse therapy has been reported in patients with pemphigus (30). Pasricha et al. described both a steroid-sparing effects and long-term remission up to 9 years (31). More than 300 pulses have been administered in our department. Side-effects are limited to facial flushing, and sleeping disturbances in the first night after administration.

The intravenous rather than the oral route is often chosen for administration of high-dose pulsed glucocorticoids, despite the lack of evidence to support the presumed necessity of intravenous administration. It is still unknown whether the effects of pulse therapy are due to the peak concentration or the time-dose effect of the high-dose steroid. We now pulse with 300 mg dexamethasone per os in stead of 1000 mg methylprednisolone or 200 mg dexamethasone intravenously. The first results, although not placebo-controlled, are promising, and will be published separately.

Azathioprine

Azathioprine is a purine-antagonist, used as first choice adjuvant immunosuppressive agent in treating pemphigus (33). The metabolism of azathioprine and 6-mercaptopurin have been more clearly defined and a mechanism for acute toxicity is identified (34). The purine antagonist azathioprine, is rapidly absorbed and methylated in the intestine to 6-mercaptopurine (6-MP), which is then metabolized in the liver and erythrocytes via three competitive pathways. The hypoxanthine phosphoribosyl transferase pathway produces several metabolites including 6-thioguanine nucleotides (6-TGNs), which are responsible for suppression of *de novo* purine synthesis and cytotoxicity. The thiopurine methyltransferase (TPMT) activity and xanthine oxidase pathways produce inactive metabolites which are excreted in the urine. TPMT shows wide interindividual variation, but xanthine oxidase does not. The TPMT pathway thus determines the drug clearance.

The drug has well-documented toxic effects on hematopoietic cells that may be acute or chronic, including: macrocytosis, anemia, trombocytopenia, leukopenia, pancytopenia, and acute bone marrow failure.

About 1 of 300 patients are homozygous for the inactive TPMT allele (35). Azathioprine should not be prescribed to such patients that will otherwise develop acute myelosuppression. TPMT as measured in erythrocytes also discloses patients with an

high TPMT activity. On standard dosage of 100-150 mg azathioprine per day, patients with an high azathioprine metabolism would be not optimal treated, and should receive more azathioprine.

We recommend to start azathioprine with a test dose of 50 mg for 3 days so that the drug can be withdrawn at early stage if gastro-intestinal complaints occur. The adequate therapeutic dosage is 1.5 or 3 mg/kg per day dependant on the TPMT-enzyme activity level of less or more than 15 U/ml. Azathioprine is continued for a period varying between 3-12 months after prednisolone is tapered to zero. Tapering azathioprine is not necessary. Daily dosage azathioprine should be halved in case of leukopenia of less than $4x10^9$/L or of if the liver enzymes increase. Azathioprine is stopped when leukopenia less than $2x10^9$/L, or when trombocytopenia is less than $100x10^9$/L.

The study of Guillaume and co-workers (36), in which 100 and 150 mg azathioprine was administered in bullous pemphigoid patients did not claim a steroid-sparing effect. TPMT was not monitored and the dose of 150 mg might have been inadequate in a number of patients. The controlled trial of Burton in 1979 (37) cumulates prednisone dosage for three years follow-up in 25 in BP patients on tapering schedule prednisone with either azathioprine (2,5 mg/kg/day), or prednisone alone. Prednisone was however "dosed on command" by the physician. Total dose of glucocorticoids was however significantly lower in the group also given azathioprine, and therefore suggests a steroid-sparing effect. Well designed placebo-controlled studies evaluating the steroid-sparing effect of azathioprine in autoimmune bullous dermatosis are lacking. Nevertheless, there is a communis opinio that azathioprine is first choice adjuvant because the low range of side-effects, compared to other immunosuppressive agents, which may have a strong efficacy but major side-effects.

Mycophenolate mofetyl

Mycophenolate mofetyl is a new immunosuppressive agent and like azathioprine, a purin-antagonist. Mycophenolate mofetyl is the semisynthetic ester of mycophenolic acid. Mycophenolic acid, the active metabolite, interferes with *de novo* synthesis of purine by inhibiting type II inosine monophosphate dehydrogenase, an enzyme expressed in proliferating of T- and B-lymphocytes.

Since mycophenolate mofetyl is less hepatotoxic than azathioprine, it might be preferable in patients with liver function abnormalities. The daily dose of 2000 mg mycophenolate mofetyl is about five times more expensive than that of 200 mg azathioprine. Enk and Knop (38;39) treated 11 of 12 PV patients successfully after first relapse on tapering schedule oral glucocorticoids with azathioprine. These patients did not relapse for a median period of 12 months. Introduction of mycophenolate mofetyl was unfortunately coined with an increase of maintenance dose prednisolone up to 2 mg/kg, so these spectacular effects are difficult to interpret (40). In another small open study, 4 pemphigus vulgaris, and 1 pemphigus foliaceus were successfully treated with adjuvant mycophenolate mofetyl (41). In 4 patients mycophenolate mofetyl was introduced after initial treatment with azathioprine (up to 250 mg/day). Also use of mycophenolate mofetyl was demonstrated to be successfully as *monotherapy* in a patient with recalcitrant PV (42). Complete remission was achieved in six weeks with 2 grams per day mycophenolate mofetyl. Since long-term (side-) effects of mycophenolate mofetyl are unknown the value of this promising immunosupressive has to be demonstrated.

Cyclophosphamide

Cyclophosphamide is an alkylating agent that disrupt cell growth and mitotic activity by cross-linking DNA. It is appears to be highly effective in maintaining remission in pemphigus. Pasricha used cyclophosphamide 500 mg i.v. (only on the first day) in conjunction with high-dose glucocorticoid pulse therapy. Besides daily 50 mg oral cyclophosphamide was administered (43). The main concern of cyclophosphamide in non-oncologic doses are hemorrhagic cystitis in particular after daily administration and increased risk of malignancy after prolonged use. Therefore pulsed cyclophosphamide was introduced for improving efficacy in combination with low-rate of side-effects. Fleischli et al. used intravenous cyclophosphamide pulses of 500 mg in nine patients, of which four responded partially and two achieved remission of skin lesions (44). However, most patients experienced severe side-effects. All patients were also on daily dosage cyclophosphamide 50 mg per day and prednisolone.

Methotrexate

Methotrexate as adjuvant therapy was initially recommended in treatment of pemphigus (27), however concerns about severe toxic effects after often required high-dosage (up to 150 mg / week) has lead to preferences for other treatment modalities (8;9). A recent study showed remission induction in six of nine chronic vulgaris patients (45), in which prednisone could not be tapered without flare-up. Disease flared-up after about 3 weeks when administration of methotrexate was discontinued. In a contrary review study, MTX was found not the reduced mortality or change the rate of remission (46).

Gold

In cases with mild or moderate pemphigus, gold compounds, sometimes used as monotherapy, can be effective. The mechanisms of action remains unexplained. Because a delayed onset of action, patients often require oral glucocorticoids for initial disease control. Usually therapy is started with 50 mg aurothioglucose i.m. once a week (after try-out dose of 10 mg). Frequence of administration is decreased to monthly injections after cumulative dose of one gram aurothioglucose. Monitor for proteinuria (nephritis) and eosinophilia.

Pandya *et al.* (47) reviewed 22 patients treated with gold and prednisone, and 4 with gold alone over a period of 10 years. The average duration of gold therapy was 12 months. Twenty patients achieved remission, lasting for an average of 8 months.

Tetracyclins and nicotinamide

In case of mild disease, first a combination of nicotinamide (1500 mg/day) and tetracyclins (2 gr/day) can be tested for a period of four weeks. Primary advantage nicotinamide and tetracyclins offer over corticosteroids (and other immunosupressives agents) is a broader safety profile. The most common side-effect is gastrointestinal upset.

Tetracyclins have an inhibitoir effect on chemotaxis of neutrophilic and eosinophilic granulocytes. Besides tetracyclins possible improve the strength of the dermo-epidermal layer. Long-term use (>1 year) causes little chance for drug-induced LE (especially in woman), autoimmune hepatitis, and hypersensitivity syndrome or DRESS (“drug rash

with eosinophilia and systemic symptoms) (48). Minocycline more than tetracyclines seems at risk for DRESS, which might have a delayed onset (49).

Nicotinamide (niacinamide, vitamin B_3) has a stabilizing effect on leukocytes and mast cells, probably by increasing adenosine 3,5 cyclic-phosphate. Advised dose is 1500 mg/day. Since the maximal dosage in one capsule goes up to 50 mg, the capsules have to be hand made by the pharmacist.

A recent study showed that only a combination of nicotinamide and tetracyclins used as only oral agent in combination with topical steroids could control two of six cases of pemphigus vulgaris (50). In the other PV patients the combination appeared to be steroid sparing. The benefits has been demonstrated for tetracyclins used as only adjuvans only in the controlled trial of Calebotta et al (51) , besides low toxicity and good safety profile were demonstrated. In case of mild oral affected patients we favor mouth washes tetracycline suspension 5% FNA (taste corrected) four times a day (52).

Human intravenous immunoglobulins (HIVIG)

Encouraging results have been reported with the use of adjuvant high dose intravenous immunoglobulins (IVIG) for treatment of patients with recalcitrant autoimmune bullous disease (53-57). Therapy consists of 400 mg/kg/day IVIG in courses of 3 to 5 consecutive days per month used as adjuvant therapy in combination with maintenance schedule prednisolone and azathioprine. In pemphigus, both therapeutic successes and failures have been reported (53). Human normal immunoglobulins are expensive: 30 grams used for a single intravenous infusion costs about $1000. Two recent case reports, confirm that low dose IVIG (40 mg/kg/day) appeared to be effective in a patient with epidermolysis bullosa aquisita (58), and a patient with recalcitrant pemphigus foliaceus (59), thereby reducing the costs of this expensive treatment considerably. The amount of intravascular IgG approximates 60 g. Infusion of 3 g instead of 30 g immunoglobulins, given a standard patient with a body weight of 75 kg, seemed to be sufficient modulating the humoral immune system in our patient. The improved cost-effectiveness of low dose IVIG therapy may push this modality forward as preferable adjuvant in pemphigus (53). However, the effect of HIVIG is transient and it is recommended for rapid action in severe cases (56).

Cyclosporine

Cyclosporine plays a minor role in the treatment of pemphigus. Both beneficial effects and therapy failures have been reported in the literature (60). Even cyclosporine mouthwash (5 ml, 500 mg cyclosporine, t.d.s. for 15 mins) was shown to have beneficial effect in orally affected patients in an anecdotal report (61). The recent randomized controlled trial of Ioannides et al. (62) (33 pemphigus patients) showed no advantage of cyclosporine, 5 mg/kg/day, in addition to daily dose prednisolone equivalent 1 mg/day. Complications were even more common in the cyclosporine group.

Plasmapheresis, immunoapheresis and photopheresis

Plasmapheresis, or plasma exchange, has been experienced-based used successfully in the treatment of pemphigus. Blood plasma is exchanged for isotonic albumen solution. It is generally recommended to combine plasmapheresis with an immunosuppressive agent to prevent rebound production of IgG. This time-consuming treatment has to be repeated in short time intervals.

The controlled clinical trial of Guillaume *et al.*, in which 19 bullous-pemphigoid patients were treated by prednisolone and ten plasma exchanges over four weeks compared to 15 patients treated only by prednisolone, suggest that plasma exchange had no additional beneficial effect above low dose steroid use (63).

Immunoapharesis is a new more specific therapeutic option, in which only the pathogenic IgG is depleted in the patients plasma. IgG autoantibodies are adsorbed on anti-human IgG affinity agarose column. It was successfully used in a patient with paraneoplastic pemphigus (64). Resynthesis of IgG autoantibodies was inhibited by postapharesis intravenous immunoglobulins (IVIG), therefore the additional effect of immunoapharesis is difficult to observe since IVIG also has an immunomodulatory potency. Immunoapharesis is even more expensive than plasmapheresis.

Photopheresis is ex-vivo radiation of leukocytes with UVA-light in conjunction with a psoralen. Among leukocytes, B-cell clones that produce IgG autoantibodies are inhibited. It has successfully been performed in a patient with recalcitrant pemphigus (65,66).

Evidence-based medicine analysis of treatment efficacy

The Cochrane Controlled Trials Register and PubMed database were used to collect drug treatment studies in pemphigus. Key words used were “pemphigus” and “trial”. There were only 4 randomized clinical trials (RCT) with a Sacket level of evidence of at least II (Table III) (66). No systematic reviews were available. It is extremely difficult to compare the results, since different criteria were used for therapeutic definitions. Meta-analysis was therefore impossible.

Table III: Randomized clinical trials for pemphigus.

Study	*Indication*	*Drugs studied*	*Experimental group (n)*	*Control group (n)*	*Endpoint*	*Outcome +/-/=*	*Level of evidence*
• Guillaume, et al. 1988 [36]. Controlled study of plasma exchange in pemphigus	PV+PF	pred.* vs. pred. and plasma exchange	19	15	4 weeks	=	II
• Ratnam, et al. 1990 [26]. Pemphigus therapy with oral prednisolone regimens. A 5-year study	PV+PF	pred. 120 mg vs. 60 mg per day	11	11	5 years	short-term + long-term =	II
• Chrysomallis, et al. 1994 [6]. Treatment of oral pemphigus vulgaris	oral PV	pred. vs. pred and cyclophosphamide vs. pred and cyclosporine	10	10 8	5 years	=	II
• Ioannides, et al. 2000 [61]. Ineffectiveness of cyclosporine as an adjuvant to corticosteroids in the treatment of pemphigus.	PV+PF	pred. and cyclosporine vs. pred.	17	16	12	=	II

*Legend: *pred.= prednisolone or prednisone, PV = pemphigus vulgaris, PF = pemphigus foliaceus*

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Chapter IIIA

Pharmacokinetics of high-dose oral and intravenous dexamethasone

Abstract

Pharmacokinetics of intravenous and oral pulsed high-dose dexamethasone were studied in four patients with pemphigus vulgaris. Doses for dexamethasone were varied from 100 to 300 mg. Serum concentrations were measured by high-performance liquid chromatographic procedure with diode assay detection. Bioavailability was assessed by comparing the areas under the serum concentration-time curves following oral administration with those of intravenous administration. Mean bioavailability of high-dose oral dexamethasone was 63.4%. Side-effects were minor and were limited to temporary facial flushing both after oral and intravenous administration. Oral administration of dexamethasone in pemphigus patients showed to be more convenient and cost-reducing than administration by the intravenous route.

Tóth GG, Kloosterman Ch, Uges DRA, Jonkman MJ. Pharmacokinetics of high-dose oral and intravenous dexamethasone. Ther Drug Monit 1999;21:532-5.

Introduction

Pulse therapy refers to discontinuous administration of very high doses corticosteroids. The choice of the corticosteroid and dosis of each pulse are not standardized, but usually are equal to 500-1000 mg methylprednisolone or 100-200 mg dexamethasone (1). The aim of this study is getting quicker and stronger efficacy and decreasing the need for long-term continuous corticosteroid use, and therefore reducing side-effects. Pulse therapy is reported to be successful in the treatment of many inflammatory or immunological diseases, i.e. cerebral edema, lupus nephritis, systemic vasculitis, severe asthma, multiple sclerosis, rheumatoid arthritis and pemphigus (1,2).

The intravenous route is often chosen for administration of high-dose pulsed corticosteroids rather than the oral route, despite the lack of evidence to support the presumed necessity of the intravenous route. Recent studies show equivalent therapeutic responses in patients with multiple sclerosis and rheumatoid arthritis after oral and intravenous administration of the same high dose of pulsed methylprednisolone (3-5).

In contrast to methylprednisolone, no efforts have been made to elucidate the pharmacokinetics of high-dose dexamethasone, despite its negligible sodium-retaining properties, low equipotent volume, and nil presystemic metabolism. It is conceivable, although not convincingly proven, that dexamethasone is less likely to cause serious cardiac dysrhytmias than methylprednisolone (6).

This study was undertaken to elucidate the pharmacokinetics of intravenous and oral high-dose pulses dexamethasone, and estimate the adequate dosis for the oral route.

Materials and Methods

Subjects

Approval for this study was obtained from the medical-ethical committee of the hospital. Informed consent was obtained from each patient. The patients were treated with prednisolone maintenance therapy in addition to dexamethasone pulse therapy every month in courses of three consecutive days.

Four pemphigus vulgaris patients were enrolled in this study. The patients comprised three males with an average age of 35.3 years (range 28-46), and mean weight of 77.5 kg

(range 74-83), and a 51 year old female with a body-weight of 92.4 kg. All patients were on daily doses of oral prednisolone 10-60 mg, ranitidine 150 mg, and a combination of etidronate and calcium carbonate (Didrokit®). One patient was also taking azathioprine 150 mg daily. Two patients suffered from a steroid-induced diabetes, requiring either insulin or tolbutamide. There were no further concomitant diseases in the medical histories. Pulse therapy was performed clinically. Every patient was given a complete medical history and physical examination. Prior and after pulse therapy safety parameters in blood (hemoglobin concentration, hematocrit, leucocytes (differentiation), trombocytes, eosinophils, liver function, kidney function, glucose) and urine (sediment, reduction) were measured. During pulse therapy every hour blood pressure and heart rate were monitored as well as blood glucose levels in the diabetic patients.

Drug administration

Patients were given 100, 200 and 300 mg dexamethasone by oral and intravenous route according to Table 1. There was no sequence of administration. The period in between each dexamethasone dose was at least 24 hours.

For intravenous administration the water-soluble ester dexamethasone phosphate was used, since dexamethasone has a low water solubility. For oral administration dexamethasone was used.

- Therapy 1: Intravenous administration over one hour of 200 mg dexamethasone-phosphate.
- Therapy 2: Intravenous administration over one hour of 100 mg dexamethasone-phosphate.
- Therapy 3: Oral administration of 200 mg dexamethasone; two gelatin capsules containing 100 mg of dexamethasone.
- Therapy 4 : Oral administration of 300 mg dexamethasone; three gelatin capsules containing 100 mg of dexamethasone.

The high-dose dexamethasone capsules were made by the hospital pharmacist, since the highest dose in tablets available in the Netherlands is only 6 mg. The patients were not fastened prior to the oral dose. The capsules were swallowed with a glass of water.

Table 1: Treatment schedule

	Patient			
	A	B	C	D
Therapy 1; *200 mg dexa i.v.*	X	X	X	X
Therapy 2; *100 mg dexa i.v.*			X	
Therapy 3; *200 mg dexa per os*	X	XX	XX	X
Therapy 4; *300 mg dexa per os*	X	X		X

Legend: 'X' represents single administration on a separate occasion, i.v.=intravenous, dexa=dexamethasone

Sample collection

Blood samples were drawn in 5 ml non-heparinised tubes during the first or the second day of a pulse course. Blood samples were drawn between 0 and 15 hours after oral and intravenous administration (Fig. I). The samples were centrifuged and the serum stored at -20 °C until further analysis. The blood samples were collected within a period of six months.

Pharmacokinetic analysis

Serum samples were analyzed for dexamethasone concentrations by a selective high-performance liquid chromatography (HPLC) procedure. Betamethasone (1 mg/L) was used as internal standard.

The chromatographic system incorporated an analytical column (Chromsphere 5C18, 250*4.6 mm), a guard column (Chrompack R.P., 10*2.1 mm), an autosampler (Merck-Hitachi, model AS-2000), a high-pressure pump (Separations, model 300) and a diode array detector (Gynkotek) at 244 nm with computer software to achieve data handling and peak integration. As mobile phase a mixture of phosphate buffer (10 mM, pH 6.9) and tetrahydrofuran (75:25) was used at a flow rate of 1.25 ml/min. One ml of serum was extracted with 6 ml diethylether after addition of 250 µl of internal standard. After shaking for 15 minutes and centrifugation for 5 minutes the water layer was frozen by -40 °C and the organic layer was transferred into a clean test tube, and evaporated to dryness under nitrogen. The residue was reconstituted in the mobile phase (200 µL) and 40 µL of aliquot was injected into the HPLC. Serum concentrations of dexamethasone were calculated by means of the ratio of its peak height to the peak height of the internal standard. The low level of quantitation was 25 µg/L. CV's (Coefficient of Variation) for

30.0 mg and 100.0 mg dexamethasone per litre of human serum was 1.2 % and 1.3 % interday, 2.0 % and 2.9 % intraday respectively.

For pharmacokinetic analysis the computer program MW/PHARM was used (7). This program fits the optimal compartment model, with the least square method, and calculates pharmacokinetic parameters.

For all administrations the time course of serum dexamethasone concentrations were characterized by a peak concentration (C_{max}), time of peak concentration (t_{max}), area under the serum-concentration time curve (AUC), and the half life ($t_{½}$). Bioavailability (*f*) of the orally administered dexamethasone dose was estimated according to equation:

$$f = \frac{AUC_{oral} * dose_{i.v.}}{AUC_{i.v.} * dose_{oral}}$$

AUC_{oral} and $AUC_{i.v.}$ are the area under the serum dexamethasone concentration time curve for the oral and i.v. doses, respectively.

In vitro dissolution of the capsules was tested following the standard method described in the European Pharmacopoeia (8). The capsules were compared to a standard (known quantity substance). After one hour the maximum dissolution was achieved.

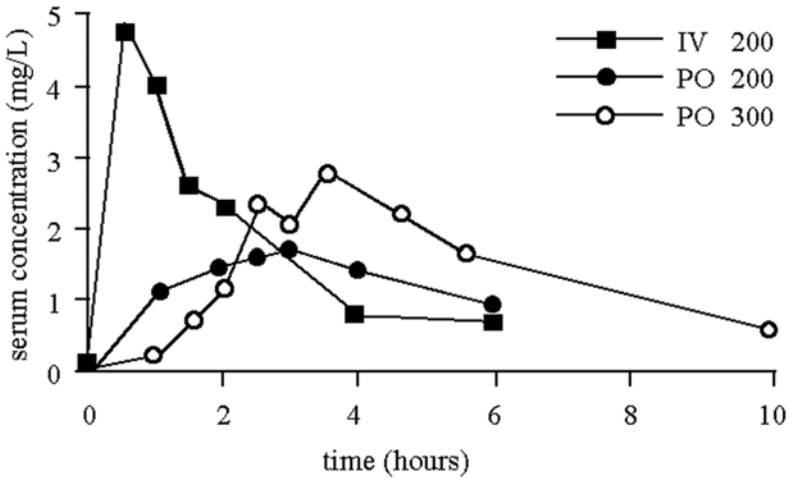
Results

During therapy no serious adverse effects occurred. In about 60 percent of the cases minor side-effects of facial flushing and sleep disturbance occurred during pulse therapy. There was no difference between intravenous and oral administration in regard to these minor side effects. Dexamethasone was not detectable 24 hours after intravenous or oral administration in any of the patients. Therapeutic effects were not relevant in this study, since the follow-up after each pulse was only 24 hours, too short to expect any effect on the skin lesions.

Figure I shows mean concentration-time curves for each administration. Here it is demonstrated that the ‘area under the curve’ after 300 mg orally approximates the same pharmacokinetic parameter after 200 mg i.v. better than after administration of 200 mg

p.o. The dexamethasone peak concentration after 300 mg oral dexamethasone (C_{max}) was 72.3% of the intravenous level at 200 mg i.v.

Figure I: Mean dexamethasone concentrations (mg/L) in all patients as a function of time (hours)



All serum concentration time data, including mean and standard deviation (S.D.), during a total of 14 cycles of treatment are summarized in table II. This table demonstrates for patient B and C the intraindividual differences in time until peak concentration (T_{max}), and peak concentration (C_{max}). Data for all doses were best described by a two compartment pharmacokinetic model using the computer program MW / PHARM.

Mean peak concentration was 4.45 mg/l after administration of 200 mg intravenously. The infusion time was one hour. The same dosis per os leaded to a mean peak concentration of 1.98 mg/l after 2.21 hours, and the mean bioavailability was 64.5%. In terms of area under curve this means that 200 mg dexamethasone per os is equivalent to 129 mg dexamethasone i.v. When 300 mg dexamethasone per os was given, mean bioavailability remained constant (61.3%), but mean peak concentration was at considerably higher level of 3.22 mg/l. Mean time to maximum concentration was 3.17 hours. Area under curve after 300 mg dexamethasone per os is equivalent to 184 mg

dexamethasone i.v. Efforts to assess the statistical significance level of the differences observed in this study were not made because of the low number of patients.

Table II: Pharmacokinetic parameters of dexamethasone after high-dose oral or intravenous therapy.

Patient	A	B1	B2	C1	C2	D	mean	s.d.
therapy 1: 200 mg dexamethasone i.v.								
AUC (h.mg/l)	16.62	17.36		10.11		15.84	14.98	3.31
C_{max} (mg/l)	4.74	4.44		3.81		4.81	4.45	0.46
$t_{½}$ (h)	2.51	2.15		0.66		3.61	2.23	1.22
therapy 2: 100 mg dexamethasone i.v.								
AUC (h.mg/l)				8.07				
C_{max} (mg/l)				2.25				
$t_{½}$ (h)				1.01				
therapy 3: 200 mg dexamethasone per os								
AUC (h.mg/l)	11.04	11.09	9.34	5.78	7.51	11.44	9.36	2.29
Bioavailability (%)	66	64	54	57	74	72	64.5	7.94
C_{max} (mg/l)	2.05	1.90	1.85	1.66	1.11	3.28	1.98	0.72
t_{max} (h)	2.50	3.00	1.25	3.00	2.50	1.00	2.21	0.87
$t_{½}$ (h)	2.66	1.62	2.06	3.09	1.09	0.78	1.88	0.90
therapy 4: 300 mg dexamethasone per os								
AUC (h.mg/l)	14.69	15.77				15.23	15.23	0.54
Bioavailability (%)	59	61				64	61.3	2.52
C_{max} (mg/l)	3.01	3.16				3.50	3.22	0.25
t_{max} (h)	3.50	3.50				2.50	3.17	0.58
$t_{½}$ (h)	1.97	2.17				1.72	1.95	0.23

Discussion

In this study mean bioavailability of oral high-dose dexamethasone was 63.4% (range 54-74%). Bioavailability levels reported in the literature for oral low-dose dexamethasone show wide variation (53-112%) (6).

In terms of AUC, oral 300 mg dexamethasone was calculated to be equivalent to 184 mg intravenous dexamethasone. Given the relative potency of methylprednisone to dexamethasone of 4: 0.75, the often clinically used intravenous dose of 1000 mg methylprednisone is equivalent to 187.5 mg dexamethasone intravenously. Since it is still unknown whether the effects of pulse therapy are due to the peak concentration (C_{max}) or the time-dose effect (AUC), 300 mg dexamethasone is the preferable dose to be used in oral pulse therapy.

No major side-effects of high-dose dexamethasone pulses were observed during this study. Furthermore, dexamethasone is cleared from the circulation within 24 hours, so that repetitive pulses on subsequent days do not lead to accumulation in the blood. Since dexamethasone has no known presystemic metabolism (9) there is no reason to worry for first-pass effects or liver damage after high-dose oral boluses. All our patients also received daily treatment with prednisolone and ranitidine. The possible influence of low dose prednisolone treatment (not detected by HPLC in this study) on high dose dexamethasone kinetics might be negligible. There is also no documented pharmacokinetic interaction between ranitidine and dexamethasone.

The clinical effects were not studied here. Recent studies however, showed no differences in efficacy and safety of oral versus i.v. pulsed high-dose corticosteroids in patients with multiple sclerosis and rheumatoid arthritis (3-5).

In conclusion, the bioavailability of high-dose dexamethasone is 63.4 percent after oral administration. Administration of 200 i.v. or 300 p.o. dexamethasone appears to be safe. This study suggests that changing the clinical use of intravenous pulses of 200 mg dexamethasone or 1000 mg methylprednisolone into oral pulses with 300 mg dexamethasone would avoid an invasive technique (vena puncture), reduces patients inconvenience, and increases cost-effectiveness, since oral intake requires less demands on medical staff and hospital room than intravenous delivery.

Acknowledgments

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Chapter III-B

Dexamethasone pharmacokinetics after high-dose oral therapy for pemphigus

To the Editor

High-dose glucocorticoid pulse therapy aims at periodically strong immunosupression, and may reduce the daily maintenance dose of glucocorticoids thus limiting the hazards of continuous long-term steroid intake (1). The high-dose glucocorticoids are administered every month on three consecutive days. Type of glucocorticoid and dose per pulse is not standardised but usually 500-1000 mg methylprednisolone per pulse, or 100-200 mg dexamethasone per pulse is administered (2). Pulse therapy is mostly given intravenously rather than orally, without evidence to support the necessity of the intravenous route. Oral therapy is preferable, avoiding vena-punctures, decreasing costs, and for patient-convenience.

Recently, the bioavailability of oral high-dose dexamethasone (100 mg capsules) of 63.4% was determined (3). In this study, the pharmacokinetics of a new dexamethasone formulation was studied, namely 50 mg tablets for oral use in pulse doses of 300 mg.

Tóth GG, Westerlaken BO, Eilders M, Laseur M, Jonkman MF, Uges DRA. Dexamethasone Pharmacokinetics After High-dose Oral Therapy for Pemphigus. Ann Pharmacother 2002;36:1109.

Methods

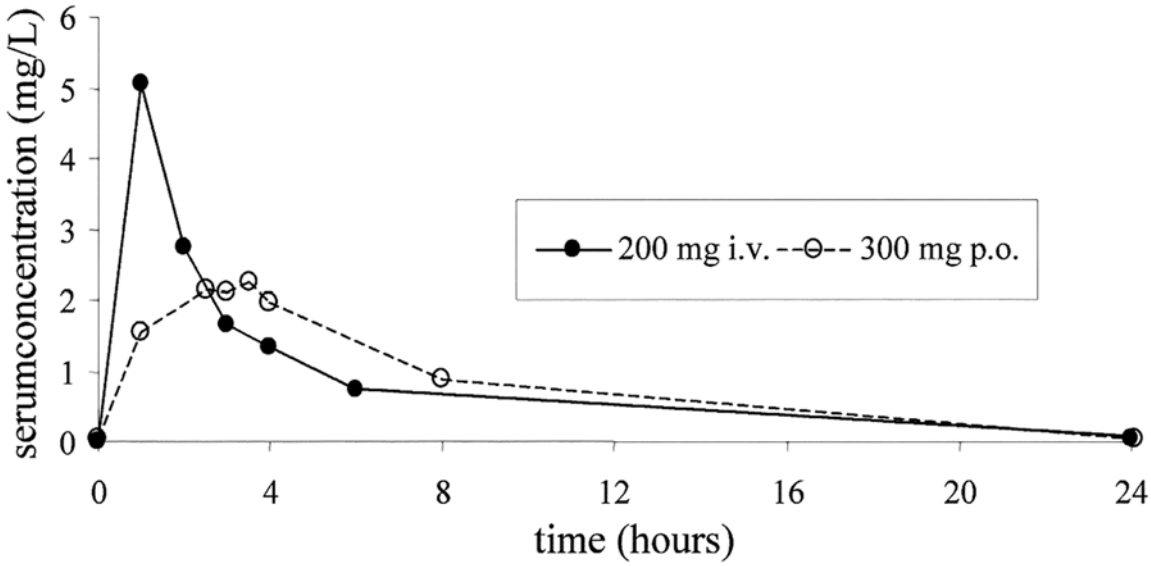
Four patients with pemphigus vulgaris currently on pulse therapy were enrolled in this study. In each patient the intravenous pulse therapy was replaced by tablets for one pulse cycle. Time in between consecutive pulses was more than 24 hours. Tablets containing 50 mg dexamethasone were produced in our hospital pharmacy. The dexamethasone (Eur. Pharm) was hydrophylised with methylcellulose 15 MPA-s (Eur. Pharm). The tablets were prepared by direct compression using cellulose mycrocrystalline (Eur. Pharm, Avicel pH101) and magnesium stearate (Eur. Pharm).

Serum samples were drawn after intravenous and oral pulse administration and analyzed by a validated suitable selective high performance liquid chromatography procedure. The same analytical procedure and extraction was used as in the previous study (see methods chapter IIIA).

Results

Figure I shows the mean serum concentration-time curve for both oral and intravenous administration of dexamethasone. The serum concentration-time curves corresponding to these administrations were best described by a tri-exponential equation. Mean bioavailability of the tablets was 55.8 % (range 43-65%). Mean peak concentration after 200 mg dexamethasone iv. was 5040 µg/L, after 300 mg dexamethasone per os 2580 µg/L. Mean time to peak concentration is 2.25 hours for oral administration.

Figure I. Mean dexamethasone serum concentrations (mg/L) in all patients a function of time (hours)



Discussion

When tablets of 50 mg were used mean dexamethasone peak concentration (C_{max}) was 52% (range 37-66%) of the intravenous level at 200 mg i.v., which is lower than after administration of the capsules (mean 72.3%; range: 67-79%) (see chapter IIIA). The dose of 358 mg dexamethasone per os is equivalent to 200 mg dexamethasone iv. Bioavailability does not differ significantly when 50 mg tablets (58.8%) or 100 mg capsules (63,4%) are used.

Since it is unknown whether the effects of pulse therapy are due to C_{max} or AUC and the bioavailability (AUC) of the tablets was comparable to capsules we concluded that 300 mg oral dexamethasone in 50 mg tablets can be used as oral pulse therapy.

Tablets are preferable instead of capsules, for better *in vitro* control of content uniformity and better taste correction. Intake of tablets is more convenient than of capsules for the patient.

The new dexamethasone tablets have reliable pharmacological and technical characteristics, and appear to be safe. They can be safely used for high-dose pulse therapy, and are suitable for use in the planned clinical trials, in which the therapeutic effect of oral high-dose dexamethasone pulse therapy is evaluated.

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3. Tóth GG, Kloosterman C, Uges DR, Jonkman MF. Pharmacokinetics of high-dose oral and intravenous dexamethasone. Ther Drug Monit 1999;21:532-5.

Chapter IV

Dexamethasone pulse therapy in pemphigus

Abstract

Pulse therapy with high-dose glucocorticoids was introduced 20 years ago as treatment modality for autoimmune disease and transplant rejection. The most popular dermatological indication for pulse therapy is severe pemphigus. We reviewed the sequelae of 14 patients with pemphigus who were treated by pulse therapy. Seven of them reached complete remission, although three of them needed a new pulse course due to disease flare-up. Adverse events were minor and confined to 60% of all patients: temporary facial flushing during pulse administration, sleep disturbances in the first night after pulse administration, and mood changes occurred during the week of pulse therapy. The study showed the possibility of oral instead of intravenous route of dexamethasone pulse administration, which makes double-blind placebo-controlled trials ethically feasible. Fifty percent of the patients reached complete remission. This retrospective study does not allow claims on the steroid-sparing effect.

Tóth GG, Jonkman MF, and van der Meer JB. Dexamethasone pulse therapy in pemphigus. J Eur Acad Dermatol Venereol 2002. In press.

Introduction

To minimalise the iatrogenic effects of cumulative steroids in pemphigus, there has been a continuous search for alternative therapies concerning treatment of this disease since 1960 (1-3). These include immunosuppressive drugs (azathioprine, cyclophosphamide, methotrexate, mycophenolate mofetil), gold, tetracyclins, dapson, intravenous immunoglobines, plasma exchange, immunoapheresis, and glucocorticoid pulse therapy. Because these treatment modalities are normally used together with, rather than instead of, oral daily systemic steroids, they can be called adjuvant therapies.

Pulse therapy, the 'big shot' (4), refers to discontinuous intravenous infusion of very high doses corticosteroids over a short time. Doses of each pulse are not standardized, but are usually 500-1000 mg methylprednisolone or 100-200 mg dexamethasone. The aim of pulse therapy is getting quicker and stronger efficacy and decreasing the need for long-term use of steroids. The contradiction seems that pulsed administration of high dose steroids is used to achieve the steroid-sparing effect. The expected steroid-sparing effect of pulse therapy has not been confidently demonstrated yet.

In the past 12 years, in six open studies exceptional benefits of glucocorticoid pulse therapy in pemphigus were reported (5-10): a high rate of complete remission and a low rate of adverse effects. A consensus regarding definitions of treatment outcome in pemphigus is lacking (1;2).

In this retrospective study, we evaluate the efficacy of pulse therapy in terms of remission, complete remission, and safety of glucocorticoid pulse therapy in a series of pemphigus patients. Mean total dose of glucocorticoids required to reach initial control, and complete remission were evaluated. We also propose unambiguous definitions for treatment outcome.

Patients and methods

The medical records of 14 patients with pemphigus treated between 1994-2000 with dexamethasone pulse therapy in the Groningen University Hospital were reviewed. One patient (#14, table I), not responding to the pulse therapy, was described previously (11). The diagnosis pemphigus was based on both clinical presentation, histopathology, and immunofluorescense characteristics.

Treatment schedule

Since 1994 the implementation of pulse therapy for pemphigus has evolved in our department. Initially, we followed the regimen of Pasricha (8): monthly 3-days courses of intravenous 100 mg dexamethasone plus a single intravenous pulse of 500 mg cyclophosphamide (DCP) in combination with low dose oral prednisolone and 50 mg oral cyclophosphamide. From 1996, we omitted cyclophosphamide from the pulse therapy (DP), because of its potential to induce neoplasm's (12;13), and because low dose cyclophosphamide may increase antibody production (14). The intravenous dose of dexamethasone was increased to 200 mg, the equivalent of 1000 mg methylprednisolone. After determining the bioavailablity of high oral dose dexamethasone (63,4%) (15), we gave since 1999 oral pulses of 300 mg dexamethasone (tablets of 50 mg, manufactured by our hospital pharmacy), instead of intravenous pulses of 200 mg. Regarding area-under-the-curve, oral pulses of 300 mg dexamethasone are equivalent to 1000 mg pulses of intravenous methylprednisolone (see chapter IIIA of this thesis). Dexamethasone is per mg about 6.7 stronger than prednisolone on the hypothalamic-adrenal axis, the mineralocorticoid effect is neglectible, and has a very low equipotent volume – strong drug fits in a small capsule or tablet – which is favorable in oral administration (16). For those inexperienced with megadosis glucocorticoids: a single pulse of 300 mg dexamethasone is equivalent to 2000 mg prednisolone. Dexamethasone was not detectable 24 hours after intravenous or oral pulse administration in a previously performed pharmacokinetic study (15), so cumulative effects are avoided when pulses are performed on consecutive days.

New pemphigus patients were treated in the same schedule: during the first week 60 mg/day prednisolone was given and pulse therapy on three consecutive days. If no initial control was reached after one week, daily dosage prednisolone was increased to 120 mg during the second week, and if necessary increased to 240 mg during third week according to Lever (17). After initial control prednisolone was tapered to 30 mg/day within 3 weeks and subsequently in 13 weeks further tapered to zero. In patients who were already on immunosupressives, prednisolone was tapered 5 mg per 2 weeks if possible. All patients had active disease when dexamethasone pulse therapy was started.

We define *active disease* in pemphigus vulgaris (PV) as more than 1 new skin lesion per week or presence of oral lesions larger than 5 mm, and presence of a positive Nikolsky's sign on non-affected skin, at least 2 cm outside an area of a pemphigus lesion (Nikolsky's sign I). The Nikolsky's sign II, c.g. on lesional skin, is important for making the diagnosis, but not for monitoring disease activity, since pemphigus lesions are always Nikolsky positive. In pemphigus foliaceus (PF), active disease was defined as more than 3 new lesions per week, and a positive Nikolsky's sign I. *Remission* is defined as no disease activity. *Initial disease control* is the first time of remission. *Complete remission* is remission without prednisolone treatment. Adjuvant medication is continued for one year after complete remission. *Relapse* is defined as re-occurrence of more than one (PV) or three (PF) new skin lesions per week, or extension of oral lesions to diameter larger than 5 mm in PV.

Prior to first pulse therapy, an extensive screenings program was performed. The first pulse administration was always given during admittance in the clinical ward. Every patient was given a complete medical history and physical examination.
Parameters for safety were measured in blood (hemoglobin concentration, hematocrit, leucocytes (differentiation), trombocytes, eosinophils, liver function, kidney function, glucose) and urine (sediment, reduction, pregnancy test in fertile female) were measured. Also an EKG, chest X-ray, and mantoux-test were performed. Blood and urine samples were repeated a week prior to the next pulse. Daily dosage prednisolone was adjusted to disease activity. Current concomitant medication was kept constantly, and interactions with dexamethasone were avoided. As prophylactic co-medication we gave all patients ranitidine 150 mg, and a combination of etidronate and calcium carbonate to prevent ulcus pepticum, and osteoporosis, respectively. During pulse therapy blood pressure and heart rate were monitored as well as blood glucose levels.

Patient demographics, and therapy prior to pulse therapy are shown in table I. For all patients the number of weeks until initial control, number of months in remission, complete remission, adverse effects, concomitant medication, and follow-up were determined from their medical records. In order to evaluate the expected corticosteroid sparing effect of pulse therapy, the mean total dose of corticosteroids required to reach

initial control, and complete remission were counted in new pemphigus patients (table III) and compared to a recent report in which corticosteroids amounts were evaluated in 33 new patients treated with only corticosteroids, or corticosteroids with adjuvant cyclosporine (18).

Results

A total of 14 patients were treated with dexamethasone pulse therapy (DP). Demographic characteristics are summarized in table I. The patients comprised 6 males and 8 females with an average age of 47.4 years old. Seven patients were diagnosed as pemphigus vulgaris, seven as pemphigus foliaceus. Seven patients were Dutch-Caucasian, five Turkish, one Dutch-Jewish, and one was Asiatic. Overall therapeutic results per patient are shown in table II. Follow-up ranges from 4-39 months. Patient 1 to 6 were newly diagnosed pemphigus patients, which did not use immunosuppressive medication prior to pulse therapy longer than one month. The other eight patients were diagnosed in an earlier stage, and therefore used long-term immunosuppressive therapy for years prior to DP. In three patients (#1, #3 and #14) a second DP-cycle had to be administered because of disease flare-up. Patient #3 had pulse therapy administered without daily prednisolone. In patient #14 DP was readministered because of relapse.

In the fourteen pemphigus patients time to remission (initial control) varied from 1 to 21 months (median 6.1 months). In newly diagnosed pemphigus patients time to first remission varied from 1 to 4 months (median 2.3 months), whereas the immunosuppressive pemphigus patients needed 4-21 months (median 9.1 months).

Time in remission varied from 0 - 21.5 months (median 8.5 months). Complete remission was established in 50% of all patients, and sustained 2-15 months within a follow-up varying from 8-39 months. Total follow-up varied from 4- 39 months (median 20.4 months). Follow-up period for new patients was shorter (median 15.8 months) than for the others (median 23.8 months). In table III cumulative corticosteroid dose is shown for the four new patients who reached complete remission. Mean prednisolone dose until initial control was 3483 mg (range 1260-6580 mg), and total prednisolone dose until complete remission was 5954 mg (range 2303-10045mg).

Table I: Demographics of pemphigus patient treated with pulse therapy in Groningen (1994-2000)

Patent/ Gender	*Onset (years)*	*Type*	*Mucosa / Skin Involvement*	*Ethnic Group*	*Previous treatment*
1/M	32	PV	+/+	Turkish	pred 4 days 120 mg
2/M	46	PV	+/+	Turkish	doxycycline 200 mg
3/M	28	PV	+/+	Turkish	none
4/F	49	PF	-/+	Dutch	pred 10 mg, aza 100
5/F	75	PF	-/+	Dutch	pred 80 mg (3 days), nicotinamide
6/M	28	PF	-/+	Dutch	nicotinamide 1500 mg, doxycycline 200mg, pred 60 mg
7/M	42	PV	+/-	Turkish	pred
8/F	51	PV	+/+	Jewish	pred
9/F	56	PV	+/+	Dutch	pred
10/F	64	PV	+/-	Dutch	tetracyline, MTX, cyclosporin, minocycline, pred up to 60 mg
11/M	21	PF	-/+	Asian	DDS, minocycline, pred 10 mg
12/F	71	PF	-/+	Dutch	pred
13/F	54	PF	-/+	Dutch	pred 7,5 mg
14/F	46	PF	-/+	Dutch	aza 150 mg, MTX 15 mg/week, hydroxychloroquine 600 mg, doxycycline 200 mg, nicotinamide 1500 mg, PE with cyclophosphamide 100 mg, pred 60 mg
Total	47.4	PV:PF 7:7			

Legend: pred = prednisolone, aza = azathioprine, PE = plasma exchange,PV = pemphigus vulgaris, PF = pemphigus foliaceus

Table III: Cumulative corticosteroid dose in pemphigus patients treated with pulse therapy

Patient	*pred until IC (mg)*	*pred until CR (mg)*
1	6580	10045
3	2730	3640
4	1260	2303
6	3360	7829
mean	3483	5954

Legend: pred = prednisolone, IC = initial control, CR = complete remission

Adverse effects

Two patients experienced serious adverse events during follow-up. One patient on dexamethasone-cyclophosphamide pulse therapy died due to metastasized bladder carcinoma. This patient had used oral cyclophosphamide for years prior to the DCP therapy. Another patient died due to a cerebrovascular accident, 10 months after stopping DP therapy. She had had been on prednisolone maintenance schedule of 10 mg or more for 19 months. There were no further serious adverse events. Diabetes was induced in 4 patients with total DP's varying from 9-16 times.

In about 60 percent of the cases minor side-effects occurred comprising: temporary facial flushing during pulse administration, sleep disturbances in the first night after pulse administration, and mood changes in the week following pulse therapy. There was no difference between intravenous and oral administration in regard to these minor side effects.

Table II: Therapeutic results of pulse therapy in pemphigus patients

Patient No.	*Adjuvant medication during DP*	*DP's No.*	*IC Months after*	*R Month*	*CR Month*	*F-U Month*	*AE*	*Status End F-U*	*Medication at end F-U (mg)*
1	pred, aza. 150 after 10 months	16	3	13	2	18	n	CR	aza. 150
2	pred, after DP 7 aza 150-200, after DP 14 gold	14	4	14	-	16	sDM	R	pred 5, aza 200, gold (total 450 mg)
3	pred, aza 150 after 10 months	10	3	7	10	20	n	CR	aza 150
4	aza 150	5	1	4	3	8	n	CR	aza 200
5	pred	9	1.5	17.5	-	19	sDM	†, R	CVA, pred 10
6	pred, aza 150, after DP 7 gold	10	1	9	4	14	n	CR	aza. 150, gold (1,1 g)
7	pred, aza 150	18	5,5	21.5	4	31	sDM	Slight flare-up	pred 20, aza 150, HIVIG
8	pred, DCP	15	6	9	-	15	x*	†, R	Bladderca.
9	pred, DCP	4	>4	-	-	4	n	TW	
10	pred, after DP 4 aza 100	9	6	6	4	16	n	CR	aza 100
11	pred, after DP 12 aza 150	22	14	17	-	31	n	R	pred, aza, HIVIG
12	pred, DCP, cyclophosfamide 50	15	7,5	3.5	15	26	n	CR	
13	pred, after DP 12 aza 50	13	9	19	-	28	sDM	R	pred 5 , aza 50, HIVIG
14	pred, cyclophosfamide 100, aza 150	31	>21	12	-	39	n	R	pred 12.5 , aza 150, HIVIG

*Legend: F-U = follow-up, IC = time to initial control, R = time in remission, CR = time in complete remission, DP = dexamethason pulse therapy, DCP = dexamethason cyclophosphamide pulse therapy F-U = follow-up, AE = adverse effect, Aza = azathioprine, HIVIG = human i.v. immunoglobulins, pred = prednisolone, TW = treatment withdrawal, n = no, y = yes, *= death after bladder carcinoma (adjuvant cyclophosphamide in pulse therapy).*

Discussion

The medical use of glucocorticoids transformed pemphigus from an almost invariably fatal disease into one whose mortality is now between 5 to 10% (2;19;20). Mortality at present is caused by the complications of long-term steroid therapy, i.e. sepsis and lung embolism (16;20;21). Therefore steroid-sparing agents still are of great interest. No consensus is available regarding initial steroid dosage needed to induce remission, and the effect of this on the subsequent course of the disease.

The aim of pulsed high-dose glucocorticoids is besides stronger efficacy, a decrease in steroid-induced side-effects. An explanation for the possible efficacy of high-dose glucocorticoids on the molecular level is lacking. A total of 191 pulses were administered in this retrospective study. Side-effects were limited to facial flushing, temporary sleeping disturbance, and mood changes. There was no difference in these side effects between oral and intravenous administration. Diabetes Mellitus was induced in 4 patients. We cannot deduce whether diabetes was induced by daily dosage corticosteroids, or by dexamethason pulse therapy. Severe adverse events related to the pulse therapy did not occur. Our results agree with those in the literature in which large placebo-controlled trials were performed in patients with ophthalmologic and neurologic disease (22;23). Severe side-effects, i.e. anaphylactic shock, seizures, sepsis, aseptic bone necrosis, cardiac arrhythmia's and sudden deaths were very rare in patients treated with high-dose steroids, and only occurring in those patients with other risk factors (24).

Complete remission was established in 50% of the patients within a maximum follow-up of 39 months. In some patients it was mandatory to start a second or a third adjuvans to sustain disease control. In 8 recalcitrant pemphigus patients, who had been on long-term steroid and/or other immunomodulators before, it turned out, to be more difficult to control disease, so that more DP's had to be administered. Three of them were in complete remission, however other adjuvant therapies were started to sustain disease control. The results of this retrospective study have to be interpreted with care, because of the uncontrolled study design, the limited number of patients, and the incomparability with other studies. Statistical efforts therefore were not made. In this study, dexamethasone pulse therapy appears to be effective to induce complete remission in

50% of the patients. The retrospective studies of Pasricha (25) and Werth (10) showed complete remission in respectively 84% and 44% of the patients. Since the treatment schedule differs between these studies, and only pemphigus vulgaris patients were included, these encouraging results are difficult to compare with our data. Nevertheless, 50% complete remission is better than rates of 30-40% reported in two reviews for patients treated with a conventional regimen of glucocorticoids (2,3). In a recent trial of Ioannides *et al.* comparing cyclosporine (5 mg/kg) as adjuvans to daily corticosteroids in 33 pemphigus patients, complete remission was achieved in 27% of the patients irrespective whether cyclosporine was added to the daily corticosteroid regimen (18). The cumulative corticosteroid use counted in prednisone-equivalents differs significantly between the 'only corticosteroid group' in the study of Ioannides *et al.* and ours: cumulative dose until initial control was 531 mg versus 3483 mg in ours, and dose until complete remission was 3385 versus 5954 in ours. As mentioned before, differences in definitions for treatment outcome were used, and a comparison is therefore difficult to make. Steroid sparing effects of pulse therapy cannot be confidently be deduced from these data. For a definite conclusion on the efficacy of dexamethasone pulse therapy in pemphigus, a double-blind placebo-controlled randomized clinical trial is needed to obtain evidence for this promising but yet to be proven treatment modality.

Acknowledgments

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Chapter V

Robust staging of disease activity for pemphigus vulgaris

Abstract

In this study a robust and simple classification into different stages of pemphigus, based on a set of therapeutic benchmarks including a definition of disease activity, is proposed. This staging method was validated against ELISA titres for autoantibodies against desmoglein-1 and -3.

The proposed staging method was applied a priori to five newly diagnosed pemphigus patients in the course of their disease. The ELISA titres for desmoglein-1 and -3, as well as the indirect immunofluorescence titres were cross tabulated against the a priori defined disease stages. ELISA titres of desmogleins seemed to show a good correlation with our proposed staging system, better than the indirect immunofluorescence titres. However, due to the small sample size, we could not perform a statistical test. We conclude that the proposed staging system for disease activity in pemphigus vulgaris is useful for international uniform monitoring pemphigus vulgaris.

Submitted for publication

Introduction

Pemphigus is a rare intraepidermal autoimmune bullous disease affecting skin and mucous membranes. Several studies propose definitions for treatment outcome such as initial control, remission, and complete remission (1-6). However, some definitions are complicated to perform, some are even conflicting with each other. A consensus for staging of disease activity in pemphigus never has been reached. Proposals for monitoring pemphigus are based on clinical parameters, such as a time-consuming counting of all blisters (1), which may be redundant for a therapeutical strategy or a scientific study. There is a need for an unambiguous easy-to-use scoring system with a small interobserver bias. Such a scoring system could be used in pemphigus trials, which often require a multicentered approach due to the low incidence of approximately 0.1-0.42 per 100.000 (7-9).

In this study such a classification into different stages of the disease, based on a set of therapeutic benchmarks including a definition of disease activity, is proposed. IgG autoantibodies to desmoglein 1 (dsg 1) and desmoglein 3 (dsg 3) were determined retrospectively by ELISA at four specific clinical therapeutic benchmarks, and compared to the indirect immunofluorescence (IF) serum titres.

Patients and methods

The medical records of 5 newly diagnosed pemphigus vulgaris patients in the Groningen University Hospital were reviewed. Diagnosis was based on clinical presentation, histological, and immunofluorescense characteristics. Patients were all on the same treatment schedule: monthly 3-days courses of 300 mg dexamethasone per os (dexamethasone-pulse = DP-therapy), or intravenous equivalent, in combination with maintenance schedule oral prednisolone, and adjuvant azathioprine 3 mg/kg/day. During the first week 80 mg prednisolone was given daily and adjuvant DP-therapy on three consecutive days. If no initial control (defined in table I) was reached after one week, daily dosage of prednisolone was increased to 120 mg during the second week, and if necessary increased to 240 mg during the third week according to Lever (10). After initial control prednisolone was tapered to 30 mg/day within 3 weeks and subsequently in 13 weeks further tapered to zero (11).

For staging pemphigus vulgaris we scored disease activity by the clinical definitions explained in table I.

Table I: Definitions for clinical staging disease activity in pemphigus vulgaris

Disease activity	*Definition*
Positive (exacerbation)	• >1 new skin lesion per week **or** oral lesions > 5 mm **and** • positive Nikolsky sign type I
Negative (remission)	• #1 new skin lesion per week **or** presence of oral lesions # 5 mm **and** • negative Nikolsky sign type I

Therapeutic Benchmarks	
Start	Start of DP-therapy
Initial Control (IC)	First remission after diagnosis and (subsequent) therapy
Complete Remission (CR)	Remission without prednisolone treatment
Relapse	Re-occurrence of positive disease activity after CR

The Nikolsky sign plays a key-role in scoring disease activity (12;13). Only Nikolsky sign type I is used: rubbing non-affected skin, at least 2 cm outside an area of a pemphigus lesion. Nikolsky sign type II, e.g. on erythematous skin, is important for making the diagnosis pemphigus, but not considered relevant for monitoring disease activity. In the definition of disease activity the development of new skin lesions and oral involvement were also included. Positive disease activity means *exacerbation*, whereas no disease activity means *remission*.

Based on the combination of disease activity and subsequent therapy, we defined four therapeutic benchmarks: start therapy, initial control, complete remission and

relapse. At these specific therapeutic benchmarks, for each patient matching sera were retrieved from our serum bank, where sera are stored at -80ºC until analysis. For these sera both indirect IF serum titres (IIF) on monkey esophagus, and anti-dsg ELISA index values (MBL, Nagoya, Japan) were determined.

Results

Patient demographics and therapy in the last months prior to admission are illustrated in table II. All patients showed erosions on both skin and oral mucosa at time of admission.

Follow-up varied from 9 to 14 months. Time to initial control varied from 1 to 4 months (median 2.6 months). Median duration of remission was 5.2 months (varying from 2 to 8 months). All patients reached complete remission. Relapse occurred in three patients respectively 2, 5, and 8 months after complete remission.

Table II: Patient demographics

Patient	*Gender*	*Age at onset*	*Mucosa / Skin Involvement*	*Previous treatment*
1	M	32	+/+	pred 4 days 120 mg/day
2	M	46	+/+	doxycycline 200 mg/day
3	M	28	+/+	none
4	F	44	+/+	pred
5	M	55	+/+	pred, aza 1.5mg/kg/day

Legend: pred = prednisolone, aza = azathioprine

A total of 20 serum samples were reviewed. Table III illustrates the relationship between the clinical benchmarks, IIF, and anti-dsg 1 & 3 ELISA values. Although wide ranges, an objective trend in titre change for each parameter is observed between consecutive benchmarks. However, there were some remarkable findings. Skin involvement in patient 4 at admission was not be accompanied by anti-dsg 1 antibodies. Initial control in patient 2 was not accompanied by decrease in indirect IF titre, however ELISA values showed a marked decrease in anti dsg 3 autoantibodies. The relapse in patient 3 was not followed by a raise of the indirect IF titre, however ELISA values showed increase for anti dsg 3 antibodies. The difference between initial control and complete remission could not be confirmed by indirect IF, nor by ELISA values in patient

5. Due to the small sample size, however, we could not perform a statistical test and thus the data are viewed only in a descriptive way.

Table III: Indirect IF (IIF) and ELISA-values (anti-dsg1 and anti-dsg 3)

	Patient 1			Patient 2			Patient 3			Patient 4			Patient 5		
	IIF	*dsg1 index value*	*dsg3 index value*	*IIF*	*dsg1 index value*	*dsg3 index value*	*IIF*	*dsg1 index value*	*dsg3 index value*	*IIF*	*dsg1 index value*	*dsg3 index value*	*IIF*	*dsg1 index value*	*dsg3 index value*
Start	640	179	194	160	82	124	320	129	171	640	2	161	640	106	150
IC	320	95	148	160	116	15	160	110	158	160	0	7	80	47	84
CR	40	18	29	40	0	1	80	0	86	10	0	0	80	8	96
Relapse	320	117	134	-	-	-	80	1	101	-	-	-	160	44	186

Legend: IIF=indirect immunofluorescence titres, IC=initial control, CR=complete remission, -= no relapse occurred, dsg = desmoglein

Index values were calculated as indicated by the manufacturer of ELISA-essay kit (MBL, Nagoya, Japan). Sera are considered positive if the index-value is above 7 (dsg 3 ELISA-kit) or 14 (dsg 1 ELISA-kit).

Discussion

This study, in spite of the limited number of patients, underscores the usefulness of a simple scoring method for staging the course of the disease: ask for new lesions, look in the mouth and perform the Nikolsky sign type I. At four consecutive therapeutic benchmarks the changes in indirect IF-, and ELISA values for anti-dsg 1 & 3 were monitored. IIF did not detect a titre-change between consecutive benchmarks in some patients, whereas a change in anti-dsg (by ELISA) was obvious (table III). This confirms the higher accuracy of the ELISA technique above indirect IF in measuring the pemphigus antibody titres (14-16).

Indirect IF is used widely since the 1960s for monitoring disease activity, but IIF pemphigus antibody titres do not always correlate well with actual disease activity (17). Indirect IF is a semi quantitative technique which is time-consuming, and these pemhigus

titres may not be available when therapeutic intervention is required. Therefore the value of indirect IF is limited for monitoring pemphigus.

Since 1997 specific autoantibodies against the ectodomains of desmoglein 1 and 3 transmembrane desmosomal adhesion proteins, can be detected by ELISA (14). Blister formation in mucous membranes involves autoantibodies against desmoglein 3 in PV, whereas blister formation in skin is currently attributed to the concomitant presence of autoantibodies against dsg 1 (18). ELISA can differentiate between these subtypes and is more time-efficient than IIF. It was demonstrated that ELISA values parallel disease activity in pemphigus (14;15). However, there are examples of a positive ELISA result, but inactive disease. Therefore therapeutic intervention based on shifts of this immunochemical assay seems to be an attractive possibility.

A positive correlation between indirect IF titres and ELISA titers was shown in 11 PV patients by Lenz et al (16). Aoyama et al. suggested using the ELISA titre of anti-dsg 1 IgG for determining the initial therapy for pemphigus foliaceus (PF) (19). PF patients with low ELISA titre may be treated with topical steroids, whereas those with high titres with glucocorticoid pulse therapy.

In this study, we demonstrate the value of a simple scoring method for staging the course of pemphigus vulgaris supported by pemphigus antibody titres as demonstrated by IIF and ELISA. The ELISA appeared to be more exact. Our simple and robust staging system may be less prone to interobserver bias, although further validation studies are needed. The staging system is easy to use and does not require time-consuming counting of the number of blisters. This staging system is currently used in a European multicenter placebo controlled trial in which the efficacy of oral high-dose dexamethasone pulse therapy is studied.

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Chapter VI

Safety of high-dose azathioprine in immunobullous patients

Summary

The most important side-effects of high-dose azathioprine are myelosuppression and liver toxicity. Since the arrival of the thiopurine methyltransferase (TPMT) assay, pancytopenia can be predicted in those patients with low enzyme levels. This also opened the opportunity to prescribe higher doses of azathioprine in the patients with normal TPMT levels. We reviewed the sequelae of 14 patients with immunobullous disease treated with high-dose azathioprine (2-3 mg/kg/day), and followed the blood cell counts and liver enzyme levels. From this study we conclude that high-dose azathioprine can safely be used in immunobullous conditions and that the assay may guard the safety of this schedule.

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Introduction

Azathioprine is for over 30 years widely used as adjuvant immunosuppressive agent to systemic corticosteroids in a variety of dermatologic conditions (1-3). Apparently good clinical response to the combination of azathioprine and prednisone was reported in pemphigus (4;5) bullous pemphigoid (6), and mucous membrane pemphigoid (7). In a randomized, not blinded, trial in bullous pemphigoid, Burton et al. found a 45% steroid-sparing effect with high dose azathioprine (2,5 mg/kg) (8). In another randomized, not blinded, trial in bullous pemphigoid, Guillaume et al. found no steroid-sparing effect with low dose azathioprine (100-150 mg) (9). Randomized placebo-controlled trials evaluating the steroid-sparing effect of azathioprine in immunobullous disease are so far lacking in the literature (10). Nevertheless, there is a communis opinio that azathioprine is first choice adjuvant in the treatment of autoimmune bullous diseases such as pemphigus, because of the low toxicity/effect ratio (1;11). The most frequent reasons that make azathioprine dose reduction necessary are myelosuppression and liver toxicity. Common practice is to prescribe low dose (50-150 mg) or to adjust azathioprine dose according to body weight only, and not to individual drug metabolism. However, the metabolism of azathioprine is individually variable and not related to the body weight (6).

In 1980 Weinshilboum and Sladek (12) revealed the pharmacokinetics of mercaptopurine, the active metabolite of azathioprine. The purine antagonist azathioprine, is rapidly absorbed and methylated in the intestine to 6-mercaptopurine (6-MP), which is then metabolized in the liver and erythrocytes via three competitive pathways. The hypoxanthine phosphoribosyl transferase pathway produces several metabolites including 6-thioguanine nucleotides (6-TGNs), which are responsible for suppression of *de novo* purine synthesis and cytotoxicity. The thiopurine methyltransferase (TPMT) activity and xanthine oxidase pathways produce inactive metabolites which are excreted in the urine. TPMT shows wide interindividual variation, but xanthine oxidase does not (6). The TPMT pathway thus determines the drug clearance.

The active metabolites of azathioprine may evoke leukopenia, occurring in 5-25% of all patients (6). In particular, patients with low or intermediate TPMT activity are

vulnerable for this hematological side-effect. Allelic polymorphism's of the TPMT gene determine the activity of the coded enzyme. Homozygotes (1 in 300) for the low activity allele are known to be at serious risk for acute myelosuppression after azathioprine intake (12). Heterozygotes with intermediate TPMT enzyme activity (11% of the patients), may be at risk for late-onset myelosuppression (6). In contrast, homozygotes for the high activity allele may be suboptimal immunosuppressed with the common standard dose of azathioprine. The TPMT profile can be assessed either by genotyping DNA or by phenotyping measuring TPMT enzyme activity in erythrocytes.

In this study we assessed the safety in terms of myelosuppression and liver toxicity of high-dose azathioprine, up to 3 mg/kg dd, in out-patient dermatological practice. The calibration of the new TPMT enzyme activity assay was determined in 152 Dutch controls. Subsequently, we determined the TPMT enzyme level and genotype on a series of immunobullous patients.

Patients and methods

14 patients with immunobullous disease were included who were treated with azathioprine and prednisone within the period 1998-2001. Azathioprine was started with a test dose of 50 mg for three days, so that the drug could be easily withdrawn at early stage if gastro-intestinal complaints would occur. Subsequently the azathioprine dose was increased to 2-3 mg/kg per day. Azathioprine dose was not tapered at the end of the treatment period and continued for 3-12 months as monotherapy after prednisone was tapered to zero.

We used the following guidelines for dose reduction in case of myelosuppression or liver toxicity. Daily dosage azathioprine was halved or reduced with 1 mg/kg in case of leukopenia of less than $4x10^9$/L, and stopped when leukopenia was less than $2x10^9$/L, or when trombocytopenia was less than $100x10^9$/L. In case the liver enzymes raised twice above high-normal value daily azathioprine dose was reduced with 50 mg and after two weeks evaluated again, and if necessary again tapered with 50 mg until the liver enzymes reached normal values. All patients received concomitant prednisolone.

Since measurement of both TPMT enzyme activity in erythrocytes and TPMT genotyping have only recently become available in our hospital, all TPMT tests were

performed after the start of the azathioprine medication. TPMT activity in erythrocytes was determined in EDTA-anticoagulated venous blood samples by HPLC-fluorometry essentially as described the group of Iven and coworkers (13;14). Hemoglobin content of cell lysates was determined with a Coulter STKS (Coulter Corporation). We used 6-ethylmercaptopurine (6-EMP) (Sigma, Holland) as an internal standard to correct for extraction losses during the extraction procedure. TPMT erythrocyte activity reference values were determined in 152 samples offered for routine haemocytometric analysis from adult patients.

Genotyping was performed as described by Yates et al (15). Polymerase chain reactions were performed to detect the G238C transversion (exon 5), the G460A (exon 7) and A719G (exon 10) transitions. Laboratory parameters, i.e. leukocytes, thrombocytes, and liver enzymes (ASAT, ALAT, γGT, LDH), were monitored at start of therapy, after one month, and than every three months. In this study, no efforts were made to assess a clinical efficacy.

Results

Figure I shows the frequency distribution of erythrocyte thiopurine methyltransferase in 152 samples offered for routine hemocytometric analysis. In this figure a bimodal distribution can be seen. The enzyme activity in the investigated samples ranged from 19.75 to 89.20 nmol methylthioguanine (6-MTG) g^{-1} $Hb.h^{-1}$, with a median of 49.96 nmol 6-MTG g^{-1} $Hb.h^{-1}$. 6-MTG, the metabolite of 6-thioguanine (6-TG) is highly fluorescent and therefore more sensitive to detect by HPLC. The break point between intermediate and high TPMT activity was set at 35 nmol 6-MTG g^{-1} $Hb.h^{-1}$.

The breakpoint between intermediate and low was set at 5 nmol 6-MTG g^{-1} $Hb.h^{-1}$. We found no samples with [C] < 5 nmol 6-MTG g^{-1} $Hb.h^{-1}$ TPMT activity.

Figure I. Frequency distribution of erythrocyte thiopurine methyltransferase activity in 152 samples offered for routine hemocytometric analysis

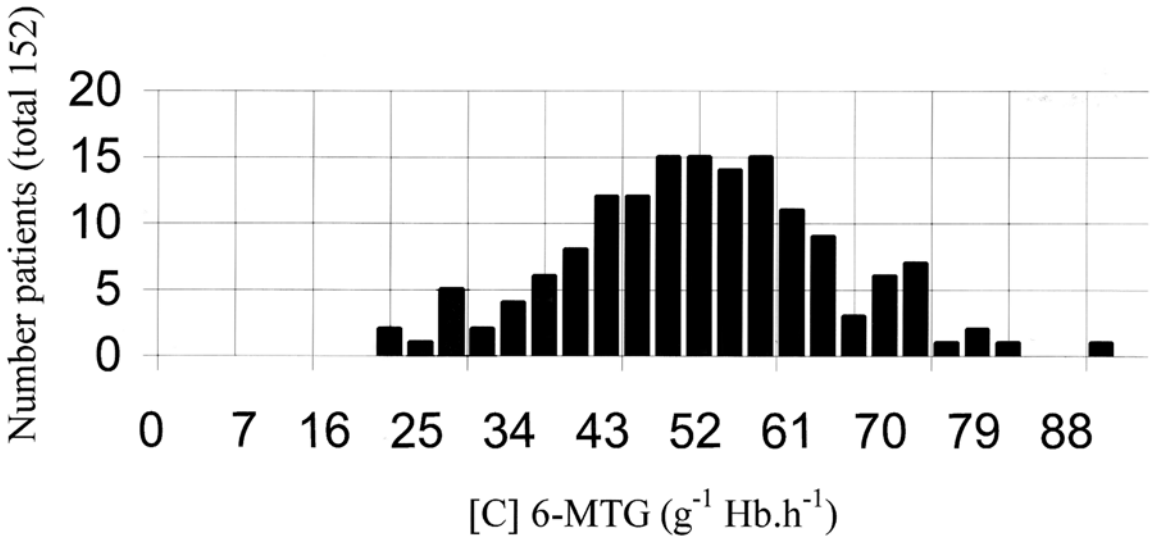

Table I lists the included patients, diagnosis, daily dose of azathioprine, TPMT phenotype, TPMT genotype, and side-effects. Two patients (#1,#2) developed leukopenia during azathioprine treatment.

In patient #1, a leukopenia of 2.9×10^9/L was encountered four months after the start of azathioprine 3 mg/kg per day. Daily dosage was tapered to 2 mg/kg per day, whereafter the leukocyte number increased to 4.3×10^9/L. After knowledge of a normal TPMT enzyme activity (55.7 nmol 6-MTG g^{-1} $Hb.h^{-1}$), azathioprine could be raised to 2.5 mg/kg per day without further hematological disturbances.

In patient #2, pretreatment leukocyte number was low at 4.0×10^9/L. Two months after medication with azathioprine 2 mg/kg per day the leukocyte number decreased to 3.2×10^9/L. Daily dosage azathioprine was stepwise reduced to 0.7 mg/kg per day until the leukocyte number was above 4.0×10^9/L. The TPMT activity was intermediate (34.0 nmol 6-MTG g^{-1} $Hb.h^{-1}$) in this patient, who was homozygous for high activity TPMT alleles. In patients #3 and #4 daily dosage azathioprine had to be decreased because of liver toxicity. A reduction azathioprine with 50 mg per patient normalized in both patients the liver enzymes. Only one patient (#5) experienced temporary mild gastro-intestinal complaints, without necessity to stop therapy. None of the 14 patients carried low activity

TPMT alleles. Nevertheless 2 patients had a TPMT level below 35 nmol 6-MTG g^{-1} $Hb.h^{-1}$. Taken together, an azathioprine dose of 2-3 mg/kg per day was well tolerated in 13 out of 14 patients.

Table I. Patients on high-dose azathioprine

No./Age/Sex	*Diagnosis*	*Azathioprin (mg/kg)*	*Duration therapy (motnhs)*	*TPMT level (phenotype) (nmol 6-MTG g^{-1} Hb h^{-1})*	*Genotype TPMT alleles*	*Leukopenia (Y/N)*	*Comment / adverse-effects*
1/67/M	MMP	3	8	55.7	Wt/Wt	Y	pretreatment L 4.9x10^9/L, after 4 months aza 3mg/kg per day, L 2.9x10^9/L,aza ↓1.5 mg/kg per day, L 4.3x10^9/L, after normal TPMT, aza ↑ 2.5 mg/kg per day, L 4.0x10^9/L
2/70/M	BP	2.5	36	34.0	Wt/Wt	Y	pretreatment L 4.0x10^9/L, after 2 months aza 2mg/kg per day, L 3.2x10^9/L, aza ↓1.5mg/kg per day, L 3.6x10^9/L, aza ↓0.7mg/kg per day, L 4.0x10^9/L
3/62/M	PV	2	3	49.8	Wt/Wt	N	Elevated asat 63, alat 66, γGT 220 Normal liverfunction at 1.5 mg/kg per day
4/24/F	PV	2	27	53.6	Wt/Wt	N	Elevated asat 92, alat 114, γGT 483 Normal liverfunction at 1.5 mg/kg per day
5/50/F	PV	3	12	38.0	Wt/Wt	N	Mild gastro-intestinal complaints
6/60/M	PV	3	18	58.0	Wt/Wt	N	
7/50/M	PV	3	24	46.1	Wt/Wt	N	
8/33/M	PV	3	40	42.2	Wt/Wt	N	
9/55/F	PV	2	60	30.6	Wt/Wt	N	
10/53/M	PF	3	24	49.9	Wt/Wt	N	
11/37/F	PF	3	28	50.9	Wt/Wt	N	
12/53/M	PF	3	3	52.0	Wt/Wt	N	
13/30/M	PF	3	49	44.4	Wt/Wt	N	
14/72/M	PF	3	25	55.4	Wt/Wt	N	

Legend: MMP = mucous membrane pemphigoid, PV = pemphigus vulgaris, PF = pemphigus foliaceus, BP = bullous pemphigoid, aza = azathioprine, Wt = wild type

Discussion

Azathioprine is generally well tolerated (16), but is known to cause severe myelosuppression in a small group of patients due to allelic polymorphism of the TPMT enzym activity (12). Common practice is to prescribe a mostly inadequate low dose azathioprine (50-150 mg per day) or to adjust the dose according to body weight only. High-dose azathioprine (2-3 mg/kg/day) is mostly reserved for recalcitrant patients. In transplantation medicine daily dose azathioprine goes up to 5 mg/kg/day (17). Using high-doses azathioprine, is nowadays guarded by determining TPMT enzyme activity. Even better is to monitor 6-thioguanine nucleotides (6-TGN), the toxic metabolites of azathioprine (17), during therapy.

In our small study population no homozygotes for low activity of TPMT were found. Of the two patients with phenotypical intermediate TPMT enzyme activity, only one patient experienced chronic leukopenia. Two patients had liver toxicity, disappearing after tapering daily dosage azathioprine.

The frequency distribution of erythrocyte thiopurine methyltransferase activity in our study group showed the bimodal distribution that can be expected on the basis of TPMT polymorphism frequency (89% high, 11% intermediate, and 0.3% low TPMT enzym activity) (12;18;19). Using the same method for TPMT enzym activity measurement, Kroplin et al. (14) found a trimodal distribution in 214 subjects. This can be explained by the difference in size of the study groups. The median TPMT enzym activity in our study group was higher than found by Kroplin *et al.*, probably due to a correction made for extraction losses by usage of an internal standard. Dosing azathioprine may possibly be done on a constant azathioprine/ TPMT activity ratio and thus further optimize the immunosuppressive effect per patient.

This studies illustrates the potential clinical benefits of elucidating the molecular basis of inherited differences in drug metabolism and disposition, and makes it feasible to more precisely select the optimal drug and dosage for individual patients. High-dose azathioprine can safely be used in immunobullous conditions when TPMT-phenotyping is performed to assess the drug tolerance of the individual patient.

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Chapter VII

Transition of pemphigus vulgaris into pemphigus foliaceus confirmed by antidesmoglein ELISA profile

Summary

We describe a case of transition from pemphigus vulgaris into pemphigus foliaceus accompanied by distinct shift in anti-desmoglein antibody profile as detected by ELISA. The patient presented with painful oral erosions, later followed by flaccid blisters and erosions on the skin. A diagnosis of pemphigus vulgaris was made based on the typical clinical presentation and histopathology, immunofluorescence, and ELISA studies. Successful therapy consisted of daily prednisolone up to 60 mg, adjuvant dexamethasone pulse therapy and azathioprine. After a period of eight months in complete remission the patient presented again with a new solitary erythematous lesion on the scalp with crusted scales. Histology showed clefting of the granular layer with acantholysis. Apparently the pemphigus vulgaris had transformed into pemphigus foliaceus. This shift from PV to PF was accompanied by the disappearance of anti-desmoglein 3 (dsg3) antibodies and the re-occurrence of anti-desmoglein 1 (dsg1) antibodies as detected by ELISA.

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Introduction

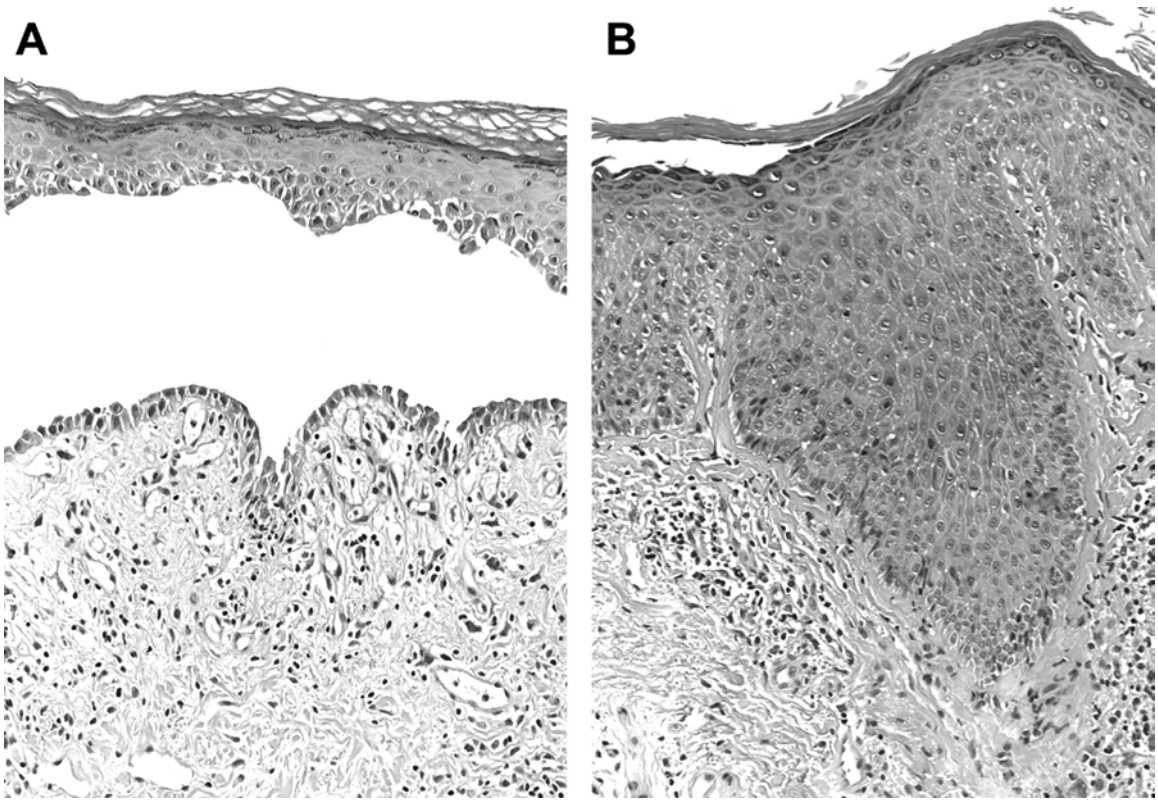
The main subtypes of pemphigus, vulgaris (PV) and foliaceus (PF), are characterized by distinct clinical, histological, and autoantibody features. Pemphigus autoantibodies are directed against the extracellular domains of the desmosomal adhesion molecules desmoglein 1 and 3 (1;2).

Transformation between the subtypes PV and PF is rarely reported in the literature. Nevertheless if such transformation does occur, then a shift from PV to PF is more common (3-9) than, vice versa, a shift from PF to PV (6;9;10). Until recently only western blot was available for determining serum antibody specificity but unfortunately immunoblotting is not very reliable in pemphigus. Due to the conformational character of part of the desmoglein epitopes many sera may give false-negative results for either desmoglein 1 or 3 or both. Recently, a transformation from PF to PV was confirmed by the desmoglein autoantibody profile as detected by ELISA (10). We present a case in which the shift from PV into PF was accompanied by disappearance of anti-desmoglein 3 and autonomous reappearance of anti-desmoglein 1, demonstrated by longitudinal follow-up.

Case report

In 1997, a 28-year-old Turkish man was referred to us with since 7 months painful lesions in the mouth, later followed by flaccid blisters and erosions on scalp, slowly spreading over the face and shoulders to the groins. Dermatological examination revealed positive Nikolsky sign's I and II. Histopathological examination of a skin lesion showed suprabasal clefting and acantholysis (Fig IA). Direct IF revealed epidermal itercellular depositions of IgG antibodies in a fishnet pattern typical for pemphigus. Indirect IF on monkey esophagus demonstrated circulating IgG antibodies against the epithelial intercellular substance (ICS) at a titre of 1:320. The ELISA assay revealed high titres of anti-desmoglein 3 (dsg3) and anti-desmoglein 1 (dsg1) IgG antibodies. A diagnosis was made of pemphigus vulgaris based on the typical clinical presentation, histopathology, IF, and ELISA studies. Figure II shows disease activity, IF titre, anti-dsg1, and anti-dsg3 titres during the course of the disease.

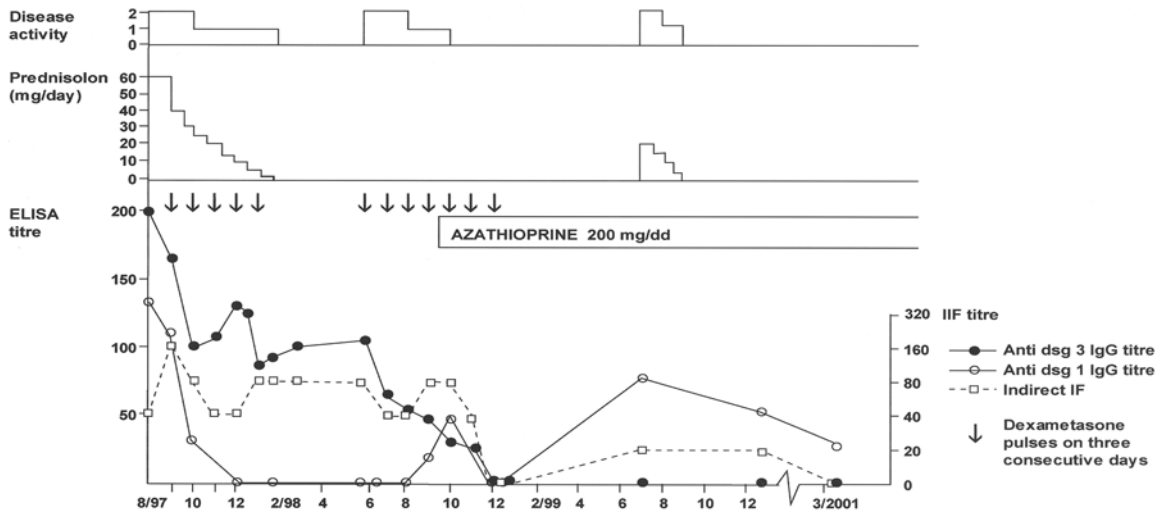
Figure I.
A.Histology at disease onset shows suprabasal acantholysis compatible with PV.
B.Histology during second relapse shows subcorneal clefting suggestive for transition in PF.



Initial therapy was started with prednisolone 60 mg per day. After one week no new blisters developed, however Nikolsky sign I (on apparently normal skin) remained positive. Therefore intravenous dexamethasone pulse therapy (DP) with 200mg dexamethasone on three consecutive days every month, was introduced. After one DP the patient reached initial control characterized by Nikolsky sign I becoming negative. A total of 5 DP's was administered. Prenisolone was tapered until zero in 5 months. Four months after complete remission he developed new blisters on the scalp, and in the mouth. DP was restarted as monotherapy, and azathioprine 200 mg per day was added to remain disease control. In this phase a total of eight DP cycles were administered. Eight months after complete remission (7/1999) the patient presented with a new solitary lesion on the scalp consisting of erythema with crusted scales. Histology showed clefting of the

granular layer, with acantholysis (Fig IB). ELISA for circulating anti-desmoglein 1 IgG gave a positive index-value of 77, whereas anti-dsg 3 was negative (Fig II). Indirect IF remained low at titre 1:20 for anti-ICS IgG. The pemphigus vulgaris had transformed into pemphigus foliaceus. Topical clobetasol dipropionate cream was applied in addittion to low-dose prednisolone without success. The patient did not suffer from the lesion on the scalp, and there was no disease progression. Treatment was not necessary.

Figure II. Disease activity, medication history, antidesmoglein 1 and antidesmoglein 3 IgG ELISA index-values, and indirect IF pemphigus titre in our patient with pemphigus during the course of disease. Disease activity is assessed on an ordinal scale with 0: no disease activity, 1: partial remission, and 2: exacerbation. ELISA index values are considered positive if exceeding 7 (desmoglein 3 ELISA) or 14 (desmoglein 1 ELISA) according the manufacturers protocol.



Discussion

Onset, progress, and transition of pemphigus depends on genetic, and exogenous factors. The development of antidesmoglein 1 antibodies in addition to antidesmoglein 3 antibodies in pemphigus vulgaris is not uncommon and correlates with disease progression from mucous membranes to skin (11). However, switching off antidesmoglein 3 antibodies in the presence of antidesmoglein 1 antibodies is uncommon and results in transition of PV into PF (2). The reason for the persistence of antidesmoglein 3 antibodies during the course of PV is unclear.

The disease period before transition may vary between 1-20 years (3). The shift from PV to PF has been reported before, in studies using immunoblot in which a shift from 130-kDa PV antigen (dsg3) to 160 kDa PF antigen (dsg1) was shown (3-5;7). Immunoblotting however, is not quantitative and often false-negative, and therefore the relation between disease activity and anti-dsg IgG titre cannot be assessed. ELISA using the ectodomains of respectively dsg1 and dsg 3 as substrates specifically measures in a quantitative way circulating autoantibodies against desmoglein 1 and 3.

A positive correlation between ELISA titers and indirect IF titers was found in 11 PV patients by Lenz et al (12). Aoyama et al. suggested to use the ELISA titre of anti-dsg 1 IgG for determining the initial therapy for PF (13). PF patients with low ELISA titre may be treated with topical steroids, whereas those with high titres with glucocorticoid pulse therapy.

Our patient developed PF with crusted scales on the scalp two years after the initial diagnosis of PV. The positive anti-dsg 1 and negative anti-dsg 3 ELISA titres, supported by the acantholysis in the upper epidermis confirmed the clinical shift into PF. Indirect IF titre remained at 1:20. In this case the ELISA values signaled us the shift in autoantibody response, and lead to a quick diagnosis, and also our choice for a milder treatment modality, since the course of PF (although sometimes chronic and refractory) is milder than in PV. Besides, this case demonstrated desmoglein 1 and 3 to change autonomously, demonstrated by the ELISA titres during a follow-up of 55 months.

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Summary

Pemphigus is a blistering autoimmune disease with autoantibodies against cell junctions between keratinocytes of the epidermis. The introduction, ***chapter I***, describes the state of the art of the pemphigus spectrum. The low annual incidence of approximately 0.3 per 100.000 per year in the Netherlands causes a sparse availability of data in the medical literature, which therefore have to be interpreted carefully. The pemphigus spectrum is subtyped based on 1) clinical presentation and histopathology, 2) subclass of autoantibodies (IgG or IgA), and 3) the targeted autoantigens. The molecular pathomechanism of pemphigus that leads to disrupting of desmosomes between keratinocytes is to a far extend elucidated. The desmosomal complex plays the major role in the cell-cell adhesion of keratinocytes and is targeted by antibodies against desmoglein. Other targets are other non-desmoglein molecular components of the keratinocyte cell membrane. Pemphigus has transformed from an almost invariably fatal disease into one whose mortality is now about 5%. However, morbidity is the main point of attention at the moment, caused by the cumulative dose of long-term used corticosteroids, necessary for sustaining disease control.

In 1997, the PEMPULS trial was designed: an international prospective, multi-centre, double-blind, placebo-controlled parallel-group, randomized clinical trial. The efficacy of adjuvant oral high-dose dexamethasone pulse therapy is studied for the so far hypothesized corticosteroid-sparing effect, and therefore contributes to the effort of adjuvant therapy in general; minimizing the iatrogenic morbidity caused by cumulative corticosteroid dose.

In ***chapter II***, current therapy in pemphigus is discussed. Systemic glucocorticoids still remain for more than 50 years the cornerstone in the treatment of pemphigus. However, they constitute a considerable health risk when used long-term. Therefore a scala of adjuvant therapies are added to the maintenance schedule corticosteroids to minimize these side-effects. No systematic reviews are available in the medical literature

to confirm steroid-sparing effects. Choice of treatment, i.e. first and second line immunomodulators, depends on disease phase. Azathioprine is considered to be first choice adjuvant because the low range of side-effects, compared to other immunosuppressive agents, which may have a strong efficacy, but major side-effects.

Chapter III describes pharmacokinetics of oral high-dose dexamethasone. Dexamethasone pulse therapy is mostly given intravenously rather than orally, without evidence to support the necessity of the intravenous route. Oral pulse therapy is preferable, avoiding vena-punctures, decreasing costs, and for patient-convenience. Pharmacokinetics of high-dose oral dexamethasone were determined to develop an oral substitute for intravenous dexamethasone pulse therapy. Bioavailability of high-dose oral dexamethasone is about 60%, and does not differ significantly when 50 mg dexamethasone tablets (58.8%) or 100 mg dexamethasone capsules (63,4%) are administered. Assuming that the effect of megadosis corticosteroid in pulstherapy is determined by the AUC and not by the peak concentration (C_{max}), we may conclude that 300 mg dexamethasone per os in 50 mg tablets can be used as oral substitute for 200 mg intravenous dexamethasone pulse therapy, which is equivalent to 1300 mg prednisolone per os. Clinical effects are studied in the PEMPULS trial, and will be reported in the near future.

Chapter IV describes the effects of dexamethasone pulse therapy in a retrospective study. A total of 207 pulses were administered in 14 pemphigus patients. Side-effects were limited to facial flushing, sleeping disturbances in the first night after administration, and mood changes. There was no difference in these side-effects between oral and intravenous administration. Severe side-effects related to the dexamethasone pulse therapy did not occur. Dexamethasone pulse therapy appeared to be effective to quicken complete remission in 50% of the patients, mainly new pemphigus vulgaris patients. A claim on the hypothesized corticosteroid-sparing effect is not allowed on basis of this retrospective open study.

In ***chapter V*** a robust and simple classification into different stages of pemphigus, based on a set of therapeutic benchmarks including a definition of disease activity, is proposed. Monitoring pemphigus is performed by scoring disease activity simply by

counting new pemphigus lesions, and using the Nikolsky sign type I. The proposed staging method was applied a-priori to five newly diagnosed pemphigus patients in the course of their disease. The ELISA titres for desmoglein-1 and -3, as well as the indirect imunofluorescence titres were crosstabulated against the a-priori defined disease stages. ELISA titres of desmogleins seemed to show a good correlation with our proposed staging system, better than the indirect imunofluorescence titres. However, due to the small sample size, we could not perform a statistical test. We conclude that the proposed staging system for disease activity in pemphigus vulgaris is useful for international uniform monitoring pemphigus vulgaris.

In ***chapter VI*** monitoring patients on high-dose (2-3 mg/kg/day) azathioprine is explained. Azathioprine has well-documented toxic acute and chronic side-effects. Since the availability of thiopurine methyltransferase (TPMT) enzyme activity test, pancytopenia due to azathioprine, can be predicted and therefore avoided. We reviewed the sequelae of 14 immunobullous patients on high-dose azathioprine and performed both TPMT phenotype (enzyme activity), and TPMT genotype. Assessing the azathioprine metabolism at an individualized level may allow a more accurate and safer choice among the different immunosuppressive modalities. From this study we conclude that high-dose azathioprine (3 mg/kg/day) can safely be used in immunobullous skin conditions.

Chapter VII describes a patient in which a transition of pemphigus vulgaris in pemphigus foliaceus occurred, accompanied by disappearance of antidesmoglein-3 antibodies, and re-occurrence of antidesmoglein-1 antibodies detected by ELISA. This study demonstrates the value of the antidesmoglein ELISA-test in the management of pemphigus.

Samenvatting

Pemphigus is een blaarvormende autoimmuunziekte met vorming van autoantilichamen gericht tegen de celverbindingen tussen huidcellen. In de introductie, ***hoofdstuk I***, wordt de huidige stand van zaken van het pemphigus spectrum besproken. De lage incidentie van ongeveer 0.3 per 100.000 per jaar in Nederland zorgt voor een spaarzame beschikbaarheid van gegevens omtrent deze ziekte in de medische literatuur, welke dan ook voorzichtig moeten worden geïnterpreteerd. Pemphigus wordt ingedeeld naar 1) de klinische presentatie en het niveau van huidsplijting, 2) de subklasse van autoantilichamen (IgG of IgA) en 3) de bijbehorende doelautoantigenen. Het moleculaire pathomechanisme dat leidt tot loslating van de verbindingen (desmosomen) tussen aaneengrenzende keratinocyten is in de loop der jaren steeds verder opgehelderd. Het desmosomale complex speelt de belangrijkste rol in de cel-cel adhesie van keratinocyten, welke wordt aangevallen door antilichamen tegen desmogleïne alswel tegen andere moleculaire componenten van de celmembraan. Pemphigus is veranderd van een dodelijke ziekte naar een ziekte waarvan de sterfte nu op ongeveer 5% ligt. Het ziekmakende effect (morbiditeit) is nu het belangrijkste punt van aandacht, welke wordt veroorzaakt door de cumulatieve dosis van de langdurig gebruikte corticosteroïden.

In 1997 werd de PEMPULS ontworpen: een internationale prospectieve, multi-centre, dubbelblinde, placebogecontroleerde parallelgroep, gerandomiseerde clinical trial. De effectiviteit van adjuvant orale hoge-dosis dexamethason puls therapie wordt onderzocht, onder meer voor het veronderstelde corticosteroïd-sparende effect, en zal daarom mogelijk bijdragen tot het doel van adjuvant therapie bij pemphigus in het algemeen; het verminderen van de iatrogene morbiditeit.

In ***hoofdstuk II*** wordt de gangbare therapie voor pemphigus behandeld. Systemische glucocorticosteroïden blijven de hoeksteen van de behandeling sinds hun introductie van meer dan 50 jaar geleden. Deze medicatie veroorzaakt echter een behoorlijke gezondheidsrisico's wanneer er sprake is van lange-termijn gebruik. Daarom wordt er een

scala aan verschillende adjuvante therapieën toegevoegd aan de dagdosis glucocorticosteroïden om deze bijwerkingen tot een minimum te beperken. In de medische literatuur zijn geen systematische reviews beschikbaar die het beoogde steroïd-sparende effect kunnen onderbouwen. Azathioprine wordt gezien als eerste keuze van adjuvante therapie, gezien het gunstige bijwerkingenprofiel vergeleken met andere immunosupressieve medicatie, welke veelal zeer effectief zijn, maar ernstige bijwerkingen kunnen hebben.

Hoofdstuk III behandelt de farmacokinetiek van hoge dosis dexamethason. Dexamethason pulstherapie wordt meestal intraveneus toegediend in plaats van oraal, zonder bewijs voor de noodzaak van de intraveneuze toedieningsvorm. Orale therapie heeft de voorkeur, wegens vermijden van venapuncties, verlagen van de kosten, en ook voor het gemak van de patiënt. De farmacokinetiek van oraal hoge dosis dexamethason pulstherapie werd bepaald om zo een oraal alternatief te ontwikkelen voor de intraveneuze toediening van pulstherapie. De biologische beschikbaarheid van hoge dosis dexamethason per os is ongeveer 60%. Deze verschilt niet significant wanneer 50 mg dexamethason tabletten (58.8%) of 100 mg dexamethason capsules (63,4%) worden gebruikt. Als wordt uitgegaan dat de effecten van corticosteroïd pulstherapie worden veroorzaakt door de totaal opgenomen daghoeveelheid (AUC: area-under-the-curve) en niet de piekconcentratie (C_{max}) kan worden geconcludeerd dat 300 mg dexamethason per os in 50 mg tabletten gelijkwaardig is met 200 mg dexamethason die via intraveneuze weg wordt toegediend. 300 mg dexamethason is qua werking gelijkwaardig met 1300 mg prednisolon per os. De klinische effecten worden bestudeerd in de PEMPULS trial, en zullen worden gerapporteerd in de nabije toekomst.

Hoofdstuk IV behandelt de effecten van dexamethason pulstherapie in een retrospectieve studie. In totaal werden 207 pulsen toegediend in 14 patiënten. Bijwerkingen bestonden slechts uit “flushing” van het gelaat, slaapstoornissen in de eerste nacht na toediening, en stemmingsveranderingen. Er werd geen verschil geconstateerd in deze bijwerkingen tussen orale of intraveneuze toediening. Ernstige bijwerkingen gerelateerd aan de dexamethason pulstherapie kwamen niet voor. Dexamethason pulstherapie leek complete remissie te bespoedigen in 50% van de

patiëntengroep, hoofdzakelijk bij nieuwe patiënten met pemphigus vulgaris. Beweringen ten aanzien van het beoogde corticosteroïd-sparende effect van pulstherapie zijn niet verantwoord op grond van deze open studie.

In ***hoofdstuk V*** wordt een robuust en eenvoudig model gepresenteerd voor de stagering van pemphigus vulgaris, gebaseerd op enkele therapeutische mijlpalen. Daarbij wordt een eenvoudige definitie voor ziekteactiviteit gehanteerd. De ziekteactiviteit wordt bepaald door de aanwezigheid van nieuwe pemphigus laesies en het teken van Nikolsky type I. De voorgestelde stageringsmethode werd a-priori toegepast op vijf nieuw gediagnostiseerde patiënten met pemphigus vulgaris tijdens het beloop van hun ziekte. De ELISA titers voor desmogleïne 1 en desmogleïne 3, als mede ook de indirecte immunofluorescencentie titers werden in een kruistabel uitgezet tegen de a a-priori gedefinieerde ziekte stadia. De ELISA titers van anti-desmogleïne IgG's bleken een goede positieve correlatie te hebben met ons pemphigus stageringssysteem, beter dan de indirecte immunofluorescentie titers. Een statistische evaluatie was wegens het lage patiëntenaantal niet gerechtvaardigd. Concluderend kunnen we stellen dat het gebruikte stageringssysteem voor ziekteactiviteit van pemphigus bruikbaar is voor internationale uniforme monitoring van pemphigus vulgaris.

In ***hoofdstuk VI*** wordt het monitoren van patiënten op hoge dosis (2-3 mg/kg/dag) azathioprine besproken. Azathioprine is bekend om toxische acute-, en chronische bijwerkingen. Sinds de beschikbaarheid van de thiopurine methyltransferase (TPMT) bepaling kan pancytopenie door azathioprine worden voorspeld. Retrospectief werden de patiëntendossiers onderzocht van 14 dermatologische immunobulleuze patiënten die ingesteld waren op hoge dosis azathioprine. Het TPMT fenotype (enzymactiviteit) en het TPMT genotype werden retrospectief bij alle patiënten bepaald. Inzicht in het azathioprine metabolisme op individueel nivo kan leiden tot veiliger maar ook meer accurater bepaling van de dosis van dit immunosuppresivum. Uit deze studie wordt geconcludeerd dat hoge dosis azathioprine (3 mg/kg/dag) veilig kan worden gebruikt bij immunobulleuze huidaandoeningen.

Hoofdstuk VII beschrijft een patiënt waarbij de transitie van pemphigus vulgaris in pemphigus foliaceus via ELISA werd herkend door het wegvallen van antidesmogleïne 3

antilichamen en aanwezigheid van antidesmoglëine 1 antilichamen. Deze studie laat de waarde zien van de anti-desmogleïne ELISA test in de diagnostiek van pemphigus.

Dankwoord

Wetenschap is leuk, tijdrovend, frustrerend, maar ook het product van samenwerken. Een aantal personen bedank ik graag persoonlijk voor de door hen geleverde bijdrage.

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Stellingen behorende bij het proefschrift

MANAGEMENT OF PEMPHIGUS

Stelling 1 Het predikaat “steroïdsparend" voor een immunosuppressief adjuvans is zonder placebo-gecontroleerd onderzoek ongegrond.

Stelling 2 Pulstherapie met 300 mg dexamethason per os is te verkiezen boven de intraveneuze toediening nu de farmakokinetiek ervan bekend is.

Stelling 3 Dexamethason pulstherapie is vanwege zijn lage bijwerkingenprofiel een aantrekkelijk adjuvans, waarvan de steroïdsparende werking nog moet worden aangetoond.

Stelling 4 De anti-desmogleïne ELISA is een specifieke en snelle bepaling, welke het monitoren van pemphigus op basis van titeruitslag mogelijk maakt.

Stelling 5 Het hanteren van een simpele definitie voor het scoren van ziekteactiviteit in pemphigus vulgaris maakt eenduidige monitoring ervan in een multicenter trial mogelijk.

Stelling 6 Generalisatie van de literatuur over pemphigus wordt beperkt door de lage incidentie van deze aandoening.

Stelling 7 Beter een pils dan een puls.

Gábor G. Tóth
Groningen, augustus 2002